

polychromatic erythrocytes micronucleus frequency in the peripheral blood was increased on day 8.

Zotti-Martelli et al. [2000] exposed human peripheral blood lymphocytes in G(0) phase to electromagnetic fields at different frequencies (2.45 and 7.7 GHz) and power densities (10, 20 and 30 mW/cm²) for 15, 30 or 60min. The results showed for both radiation frequencies an induction of micronuclei as compared to control cultures at a power density of 30mW/cm² and after an exposure of 30 and 60 min.

Zotti-Martelli et al. [2005] exposed whole blood samples from nine different healthy donors for 60, 120 and 180 min to continuous-wave 1800-MHz microwaves at power densities of 5, 10 and 20 mW/cm². A statistically significant increase of micronucleus in lymphocytes was observed dependent on exposure time and power density. A considerable decrease in spontaneous and induced MN frequencies was measured in a second experiment.

III B. Micronucleus studies that reported no significant effects:

Bisht et al. [2002] exposed C3H 10T $\frac{1}{2}$ cells to 847.74 MHz CDMA (3.2 or 4.8 W/kg) or 835.62 MHz FDMA (3.2 or 5.1 W/kg) RFR for 3, 8, 16 or 24 h. No exposure condition was found to result in a significant increase relative to sham-exposed cells either in the percentage of binucleated cells with micronuclei or in the number of micronuclei per 100 binucleated cells.

Juutilainen et al. [2007] found no significant change in micronucleus frequency in erythrocytes of mice after long-term exposure to various mobile phone frequencies.

Koyama et al. [2004] exposed Chinese hamster ovary (CHO)-K1 cells to 2450-MHz microwaves for 2 h at average specific absorption rates (SARs) of 5, 10, 20, 50, 100, and 200 W/kg. Micronucleus frequency in cells exposed at SARs of 100 and 200 W/kg were significantly higher when compared with sham-exposed controls. They speculated that the effect observed was a thermal effect.

Port et al. [2003] reported that exposure of HL-60 cells to EMFs 25 times higher than the ICNIRP reference levels for occupational exposure did not induce any significant changes in apoptosis, micronucleation, abnormal morphologies and gene expression.

Scarfi et al [2006] exposed human peripheral blood lymphocytes to 900 MHz GSM signal at specific absorption rates of 0, 1, 5 and 10 W/kg peak values. No significant change in micronucleus frequency was observed.

Vijayalaximi et al. [1997a] exposed human blood to continuous-wave 2450- MHz microwaves, either continuously for a period of 90 min or intermittently for a total exposure period of 90 min (30 min on and 30 min off, repeated three times). The mean power density at the position of the cells was 5.0 mW/cm² and mean specific absorption rate was 12.46 W/kg. There were no significant differences between RFR-exposed and sham-exposed lymphocytes with respect to; (a) mitotic indices; (b) incidence of cells showing chromosome damage; (c) exchange aberrations; (d) acentric fragments; (e) binucleate lymphocytes, and (f) micronuclei.

Vijayalaximi et al. [1997b] exposed C3H/HeJ mice for 20 h/day, 7 days/week, over 18 months to continuous-wave 2450 MHz microwaves at a whole-body average specific absorption rate of 1.0 W/kg. At the end of the 18 months, peripheral blood and bone marrow smears were examined for the extent of genotoxicity as indicated by the

presence of micronuclei in polychromatic erythrocytes. The results indicate that the incidence of micronuclei/1,000 polychromatic erythrocytes was not significantly different between groups exposed to RF radiation and sham-exposed groups.

Vijayalaximi et al. [1999] exposed CF-1 male mice to ultra-wideband electromagnetic radiation (UWBR) for 15 min at an estimated whole-body average specific absorption rate of 37 mW/kg. Peripheral blood and bone marrow smears were examined to determine the extent of genotoxicity, as assessed by the presence of micronuclei (MN) in polychromatic erythrocytes (PCE). There was no evidence for excess genotoxicity in peripheral blood or bone marrow cells of mice exposed to UWBR.

Vijayalaximi et al. [2001a] reported that there was no evidence for the induction of micronuclei in peripheral blood and bone marrow cells of rats exposed for 24h to 2450-MHz continuous-wave microwaves at a whole body average SAR of 12 W/kg.

Vijayalaximi et al. [2001b] reported that there is no evidence for the induction of chromosomal aberrations and micronuclei in human blood lymphocytes exposed in vitro for 24 h to 835.62 MHz RF radiation at SARs of 4.4 or 5.0 W/kg.

Vijayalaximi et al. [2001c] reported no evidence for induction of chromosome aberrations and micronuclei in human blood lymphocytes exposed in vitro for 24 h to 847.74 MHz RF radiation (CDMA) at SARs of 4.9 or 5.5 W/kg.

Vijayalaximi et al. [2003] exposed timed-pregnant Fischer 344 rats (from nineteenth day of gestation) and their nursing offspring (until weaning) to a far-field 1.6 GHz Iridium wireless communication signal for 2 h/day, 7 days/week at power density of 0.43 mW/cm² and whole-body average specific absorption rate of 0.036 to 0.077 W/kg (0.10 to 0.22 W/kg in the brain). This was followed by chronic, head-only exposures of male and female offspring to a near-field 1.6 GHz signal for 2 h/day, 5 days/week, over 2 years. Near-field exposures were conducted at an SAR of 0.16 or 1.6 W/kg in the brain. At the end of 2 years, all rats were necropsied. Bone marrow smears were examined for the extent of genotoxicity, assessed from the presence of micronuclei in polychromatic erythrocytes. There was no evidence for excess genotoxicity in rats that were chronically exposed to 1.6 GHz microwaves compared to sham-exposed and cage controls.

Zeni et al. [2003] investigated the induction of micronucleus in human peripheral blood lymphocytes after exposure to electromagnetic fields at various duration of exposure, specific absorption rate (SAR), and signal [continuous-wave (CW) or GSM (Global System of Mobile Communication)-modulated signal]. No statistically significant difference was detected in any case.

IV. Chromosome and genome effects (21 studies total: 13 reported effects (62%) and 8 reported no significant effect (38%))

IV A. Chromosome and genome studies that reported effects:

Belyaev et al. [1992] studied the effect of low intensity microwaves on the conformational state of the genome of X-irradiated *E. coli* cells by the method of viscosity anomalous time dependencies. A power density of 1 microW/cm² is sufficient to suppress radiation-induced repair of the genome conformational state.

- Belyaev et al. [1996] studied the effect of millimeter waves on the genome conformational state of *E. coli* AB1157 by the method of anomalous viscosity time dependencies in the frequency range of 51.64-51.85 GHz. Results indicate an electron-conformational interactions.
- Belyaev et al. [2005] investigated response of lymphocytes from healthy subjects and from persons reporting hypersensitivity to microwaves from GSM mobile phone (915 MHz, specific absorption rate 37 mW/kg), and power frequency magnetic field (50 Hz, 15 microT peak value). Changes in chromatin conformation were measured with the method of anomalous viscosity time dependencies (AVTD). Exposure at room temperature to either 915 MHz or 50 Hz resulted in significant condensation of chromatin, shown as AVTD changes, which was similar to the effect of heat shock at 41 degrees C. No significant differences in responses between normal and hypersensitive subjects were detected.
- Belyaev et al. [2006] investigated whether exposure of rat brain to microwaves of global system for mobile communication (GSM) induces DNA breaks, changes in chromatin conformation and in gene expression at a specific absorption rate (SAR) of 0.4 mW/g for 2 h. Data showed that GSM MWs at 915 MHz did not induce DNA double stranded breaks detectable by pulsed-field gel electrophoresis or changes in chromatin conformation, but affected expression of genes in rat brain cells.
- Gadhia et al. [2003] reported a significant increase in dicentric chromosomes in blood cells among mobile users who were smoker–alcoholic as compared to nonsmoker–nonalcoholic; the same held true for controls of both types.
- Garaj-Vrhovac et al. [1990] exposed V79 Chinese hamster cells to continuous-wave 7.7 GHz RFR at power density of 30 mW/cm² for 15, 30, and 60 min. Results suggest that the radiation causes changes in the synthesis as well as in the structure of DNA molecules.
- Garaj-Vrhovac et al. [1991] exposed V79 Chinese hamster fibroblast cells to continuous wave 7.7 GHz radiation at power density of 0.5 mW/cm² for 15, 30 and 60 min. There was a significantly higher frequency of specific chromosome aberrations such as dicentric and ring chromosomes in irradiated cells.
- Mashevich et al. [2003] found that human peripheral blood lymphocytes exposed to continuous 830-MHz electromagnetic fields (1.6-8.8 W/kg for 72 hr) showed a SAR-dependent chromosome aneuploidy, a major “somatic mutation” leading to genomic instability and thereby to cancer. The aneuploidy was accompanied by an abnormal mode of replication of the chromosome 17 region engaged in segregation (repetitive DNA arrays associated with the centromere), suggesting that epigenetic alterations are involved in the SAR dependent genetic toxicity. The effects were non-thermal.
- Ono et al. (2004) exposed pregnant mice intermittently at a whole-body averaged specific absorption rate of 0.71 W/kg (10 seconds on, 50 seconds off which is 4.3 W/kg during the 10 seconds exposure) for 16 hours a day, from the embryonic age of 0 to 15 days. At 10 weeks of age, mutation frequencies at the lacZ gene in spleen, liver, brain, and testis were examined. Quality of mutation assessed by sequencing the nucleotides of mutant DNAs revealed no appreciable difference between exposed and non-exposed samples.
- Sarimov et al. [2004] reported that exposure to microwaves of 895-915 MHz at 5.4 mW/kg resulted in statistically significant changes in condensation of chromatin in

human lymphocytes. Effects are similar to stress response, differ at various frequencies, and vary among donors.

Sarkar et al. [1994] exposed mice to 2450-MHz microwaves at a power density of 1 mW/cm² for 2 h/day over a period of 120, 150 and 200 days. Rearrangement of DNA segments were observed in testis and brain of exposed animals.

Semin et al. [1995] exposed DNA samples at 18°C at 10 different microwave frequencies (4- to 8 GHz, 25 ms pulses, 0.4 to 0.7 mW/cm² peak power, 1- to 6-Hz repetition rate, no heating). Irradiation at 3 or 4 Hz and 0.6 mW/cm² peak power clearly increased the accumulated damage to the DNA secondary structure ($P < .00001$). However, changing the pulse repetition rate to 1, 5, 6 Hz, as well as changing the peak power to 0.4 or 0.7 mW/cm² did not induce significant effect. Thus, the effect occurred only within narrow 'windows' of the peak intensities and modulation frequencies.

Sykes et al. [2001] exposed mice daily for 30 min to plane-wave fields of 900 MHz with a pulse repetition frequency of 217 Hz and a pulse width of 0.6 ms for 1, 5 or 25 days. Three days after the last exposure, spleen sections were screened for DNA inversion events. There was no significant difference between the control and treated groups in the 1- and 5-day exposure groups, but there was a significant reduction in inversions below the spontaneous frequency in the 25-day exposure group. This observation suggests that exposure to RF radiation can lead to a perturbation in recombination frequency which may have implications for recombination repair of DNA.

IV. B. Chromosome and genome studies that reported no significant effects:

Antonopoulos et al. [1997] found no significant change in cell cycle progression and the frequencies of sister-chromatid exchanges in human lymphocytes exposed to electromagnetic fields of 380, 900 and 1800 MHz.

Ciaravino et al. [1991] reported that RFR did not affect changes in cell progression caused by adriamycin, and the RFR did not change the number of sister chromatid exchanges that were induced by the adriamycin.

Garson et al. [1991] analyzed lymphocytes from Telecom Australia radio-linemen who had all worked with RFR in the range 400 kHz-20 GHz with exposures at or below the Australian occupational limits. There was no significant increase in chromosomal damage in circulating lymphocytes.

Gos et al. [2000] exposed actively growing and resting cells of the yeast *Saccharomyces cerevisiae* to 900-MHz Global System for Mobile Communication (GSM) pulsed modulation format signals at specific absorption rates (SAR) of 0.13 and 1.3 W/kg. They reported no significant effect of the fields on forward mutation rates on the frequency of petite formation, on rates of intrachromosomal deletion formation, or on rates of intragenic recombination in the absence or presence of the genotoxic agent methyl methanesulfonate.

Kerbacher et al (1990) reported that exposure to pulsed 2450-MHz microwaves for 2 h at an SAR of 33.8 W/kg did not significantly cause chromosome aberrations in CHO cells. The radiation also did not interact with Mitomycin C and Adriamycin.

Komatsubara et al. [2005] reported that exposure to 2.45-GHz microwaves for 2 h with up to 100 W/kg SAR CW and an average 100 W/kg PW (a maximum SAR of 900 W/kg) did not induce chromosomal aberrations in mouse m5S cells.

Meltz et al. [1990] reported no significant mutagenic effect of exposure to 2.45-GHz RFR (40 W/kg) alone and interaction with proflavin, a DNA-intercalating drug, in L5178Y mouse leukemic cells.

Roti-Roti et al. [2001] reported no significant effect of exposure to radiofrequency radiation in the cellular phone communication range (835.62 MHz frequency division multiple access, FDMA; 847.74 MHz code division multiple access, CDMA) on neoplastic transformation frequency using the in vitro C3H 10T(1/2) cell transformation assay system.

Takahashi et al. [2002] exposed mice to 1.5 GHz EMF in the head region at 2.0, 0.67, and 0 W/kg specific absorption rate for 90 min/day, 5 days/week, for 4 weeks. No mutagenic effect in mouse brain cells was detected.

V. Conclusions

From this literature survey, since only 50% of the studies reported effects, it is apparent that there is no consistent pattern that radiofrequency radiation exposure could induce genetic damages/changes in cells and organisms. However, one can conclude that under certain conditions of exposure, radiofrequency radiation is genotoxic. Data available are mainly applicable only to cell phone radiation exposure. Other than the study by Phillips et al [1998], there is no indication that RFR at levels that one can experience in the vicinity of base stations and RF-transmission towers could cause DNA damage.

During cell phone use, a relatively constant mass of tissue in the brain is exposed to the radiation at relatively high intensity (peak SAR of 4 - 8 W/kg). Several studies reported DNA damage at lower than 4 W/kg. This questions the wisdom of the IEEE Committee in using 4 W/kg as the threshold of effect for exposure-standard setting. Furthermore, since critical genetic mutations in one single cell are sufficient to lead to cancer and there are millions of cells in a gram of tissue, it is inconceivable that the base of SAR standard was changed from averaged over 1 gm of tissue to 10 gm. (The limit of localized tissue exposure has been changed from 1.6 W/kg averaged over 1 gm of tissue to 2 W/kg over 10 gm of tissue. Since distribution of radiofrequency energy is non-homogenous inside tissue, this change allows a higher peak level of exposure.) What actually needed is a better refinement of SAR calculation to identify 'peak values' of SAR inside the brain,

Aside from influences that are not directly related to experimentation [Huss et al., 2007], many factors could influence the outcome of an experiment in bioelectromagnetics research.

Any effect of EMF has to depend on the energy absorbed by a biological entity and on how the energy is delivered in space and time. Frequency, intensity, exposure duration, and the number of exposure episodes can affect the response, and these factors can interact with each other to produce different effects. In addition, in order to understand the biological consequence of EMF exposure, one must know whether the effect is cumulative, whether compensatory responses result, and when homeostasis will break down. The contributions of these physical factors are discussed in a talk presented in

Vienna, Austria in 1998. The paper is posted in many websites (e.g., <http://www.wave-guide.org/library/lai.html>).

Thus, differences in outcomes of the research on genotoxic effects of RFR could be explained by the many different exposure conditions used in the studies. An example is the study of Phillips et al. [1998] showing that different cell phone signals could cause different effects on DNA (i.e., an increase in strand breaks with exposure to one type of signal and a decrease with another). This is further complicated by the fact that some of the studies listed above used very poor exposure procedures with very limited documentation of exposure parameters, e.g., using a cell phone to expose cells and even animals. Data from these experiments are questionable.

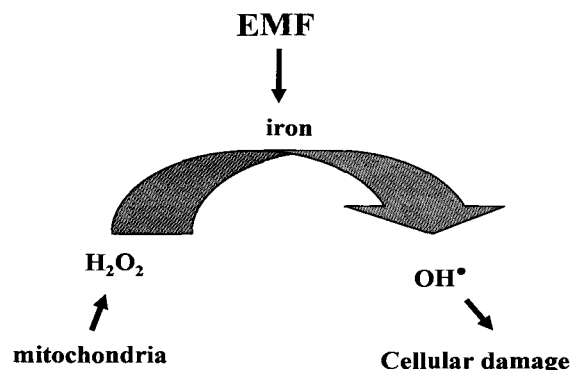
Another source of influence on an experimental outcome is the cell or organism studied. Many different biological systems were used in the genotoxicity studies. Different cell types [Hoyto et al., 2007] and organisms [Anderson et al., 2000; DiCarlo and Litovitz, 1999] may respond differently to EMF.

A few words have to be said on the 'comet assay', since it was used in most of the EMF studies to determine DNA damage. Different versions of the assay have been developed. These versions have different detection sensitivities and can be used to measure different aspects of DNA strand breaks. A comparison of data from experiments using different versions of the assay may be misleading. Another concern is that most of the 'comet assay' studies were carried out by experimenters who had no prior experience on the assay. My experience with the 'comet assay' is that it is a very sensitive assay and requires great care in performing. Thus, different detection sensitivities could result from different experimenters, even following the same procedures. One way to solve this experimental variation problem is for each researcher or laboratory to report their sensitivity of the 'comet assay', e.g., threshold of detecting strand breaks in human lymphocytes exposed to x-rays. This information is generally not available from the EMF-genotoxicity studies. However, in one incidence, an incredibly high sensitivity was even reported [Malyapa et al., 1998], suggesting the inexperience of the researchers on the assay.

A drawback in the interpretation and understanding of experimental data from bioelectromagnetic research is that there is no general acceptable mechanism on how EMF affects biological systems. The mechanism by which RFR causes genetic effect is unknown. Since the energy level is not sufficient to cause direct breakage of chemical bonds within molecules, the effects are probably indirect and secondary to other induced-chemical changes in the cell.

One possibility is via free radical formation inside cells. Free radicals kill cells by damaging macromolecules, such as DNA, protein and membrane. Several reports have indicated that electromagnetic fields (EMF) enhance free radical activity in cells [e.g., Lai and Singh, 1997a, b; 2004; Oral et al., 2006; Simko, 2007], particularly via the Fenton reaction [Lai and Singh, 2004]. The Fenton reaction is a catalytic process of iron

to convert hydrogen peroxides, a product of oxidative respiration in the mitochondria, into hydroxyl free radical, which is a very potent and toxic free radical.



THE FENTON REACTION

What is interesting is that extremely-low frequency EMF has also been shown to cause DNA damage (see the list of papers on ELF EMF and DNA at the end of this chapter). Free radicals have also been implicated in this effect of ELF EMF. This further supports the view that EMF affects DNA via an indirect secondary process, since the energy content of ELF EMF is much lower than that of RFR.

Effects via the Fenton reaction predict how a cell would respond to EMF:

1. Cells that are metabolically active would be more susceptible to the effect because more hydrogen peroxide is generated by the mitochondria to fuel the reaction.
2. Cells that have a high level of intracellular free iron would be more vulnerable. Cancer cells and cells undergoing abnormal proliferation have a high concentration of free iron because they uptake more iron and have less efficient iron storage regulation. Thus, these cells could be selectively damaged by EMF, and EMF could potentially be used for the treatment of cancer and hyperplasia diseases. The effect could be further enhanced if one could shift anaerobic glycolysis of cancer cells to oxidative glycolysis. There is quite a large database of information on the effects of EMF (mostly in the ELF range) on cancer cells and tumors. The data tend to indicate that EMF could retard tumor growth and kill cancer cells.
3. Since the brain is exposed to rather high levels of EMF during cell phone use, the consequences of EMF-induced genetic damage in brain cells are of particular importance. Brain cells have a high level of iron. Special molecular pumps are present on nerve cell nucleus membrane to pump iron into the nucleus. Iron atoms have been found to intercalate within DNA molecules. In addition, nerve cells have a low capability for DNA repair and DNA breaks could accumulate. Another concern is the presence of superparamagnetic iron-particles (magnetites) in body tissues,

particularly in the brain. These particles could enhance free radical activity in cells and cellular-damaging effects of EMF. These factors make nerve cells more vulnerable to EMF. Thus, the effect of EMF on DNA could conceivably be more significant on nerve cells than on other cell types of the body. Since nerve cells do not divide and are not likely to become cancerous, more likely consequences of DNA damage in nerve cells are changes in functions and cell death, which could either lead to or accelerate the development of neurodegenerative diseases. Double strand breaks, if not properly repaired, are known to lead to cell death. Cumulative DNA damage in nerve cells of the brain has been associated with neurodegenerative diseases, such as Alzheimer's, Huntington's, and Parkinson's diseases. However, another type of brain cells, the glial cells, can become cancerous, resulting from DNA damage. The question is whether the damaged cells would develop into tumors before they are killed by EMF due to over accumulation of genetic damages. The outcome depends on the interplay of these different physical and biological factors: an increase, decrease, or no significant change in cancer risk could result.

4. On the other hand, cells with high antioxidant potentials would be less susceptible to EMF. These include the amount of antioxidants and anti-oxidative enzymes in the cells. Furthermore, the effect of free radicals could depend on the nutritional status of an individual, e.g., availability of dietary antioxidants, consumption of alcohol, and amount of food consumption. Various life conditions, such as psychological stress and strenuous physical exercise, have been shown to increase oxidative stress and enhance the effect of free radicals in the body. Thus, one can also speculate that some individuals may be more susceptible to the effects of EMF exposure.

More research has to be carried out to prove the involvement of the free radicals in the biological effects of EMF. However, the Fenton reaction obviously can only explain some the genetic effects observed. For example, RF- and ELF EMF-induced DNA damages have been reported in normal lymphocytes, which contain a very low concentration of intracellular free iron.

This page is intentionally left blank

VI. References for Radiofrequency Radiation Studies

Aitken RJ, Bennetts LE, Sawyer D, Wiklendt AM, King BV. Impact of radio frequency electromagnetic radiation on DNA integrity in the male germline. *Inter J Androl* 28:171-179, 2005.

Anderson LE, Morris JE, Sasser LB, Löschner W. Effects of 50- or 60-hertz, 100 microT magnetic field exposure in the DMBA mammary cancer model in Sprague-Dawley rats: possible explanations for different results from two laboratories. *Environ Health Perspect.* 108(9):797-802, 2000.

Antonopoulos A, Eisenbrandt H, Obe G, Effects of high-frequency electromagnetic fields on human lymphocytes in vitro. *Mutat Res* 395(2-3): 209-214, 1997.

Balode, Z, Assessment of radio-frequency electromagnetic radiation by the micronucleus test in bovine peripheral erythrocytes. *Sci Total Environ* 180(1):81-85, 1996.

Belyaev IYa, Alipov YD, Shcheglov VS, Lystsov VN, Resonance effect of microwaves on the genome conformational state of E. coli cells. *Z Naturforsch [C]* 47(7-8):621-827, 1992.

Belyaev IY, Shcheglov VS, Alipov YD, Polunin VA, Resonance effect of millimeter waves in the power range from 10(-19) to 3 x 10(-3) W/cm2 on Escherichia coli cells at different concentrations. *Bioelectromagnetics* 17(4):312-321, 1996.

Belyaev IY, Hillert L, Protopopova M, Tamm C, Malmgren LO, Persson BR, Selivanova G, Harms-Ringdahl M. 915 MHz microwaves and 50 Hz magnetic field affect chromatin conformation and 53BP1 foci in human lymphocytes from hypersensitive and healthy persons. *Bioelectromagnetics.* 26(3):173-184, 2005.

Belyaev IY, Koch CB, Terenius O, Roxstrom-Lindquist K, Malmgren LO, H Sommer W, Salford LG, Persson BR. Exposure of rat brain to 915 MHz GSM microwaves induces changes in gene expression but not double stranded DNA breaks or effects on chromatin conformation. *Bioelectromagnetics.* 27:295-306, 2006.

Bisht KS, Moros EG, Straube WL, Baty JD, Roti Roti JL, The Effect of 835.62 MHz FDMA or 847.74 MHz CDMA Modulated Radiofrequency Radiation on the Induction of Micronuclei in C3H 10T½ Cells. *Radiat. Res.* 157, 506–515, 2002.

Busljeta I, Trosic I, Milkovic-Kraus S. Erythropoietic changes in rats after 2.45 GJz nonthermal irradiation. *Int J Hyg Environ Health.* 207(6):549-554, 2004.

Chang SK, Choi JS, Gil HW, Yang JO, Lee EY, Jeon YS, Lee ZW, Lee M, Hong MY, Ho Son T, Hong SY. Genotoxicity evaluation of electromagnetic fields generated by 835-MHz mobile phone frequency band. *Eur J Cancer Prev.* 14(2):175-179, 2005.

Ciaravino V, Meltz ML, Erwin DN, Absence of a synergistic effect between moderate-power radio-frequency electromagnetic radiation and adriamycin on cell-cycle progression and sister-chromatid exchange. *Bioelectromagnetics* 12(5):289-298, 1991.

d'Ambrosio G, Massa R, Scarfi MR, Zeni O, Cytogenetic damage in human lymphocytes following GSMK phase modulated microwave exposure. *Bioelectromagnetics* 23:7-13, 2002.

Di Carlo AL, Litovitz TA. Is genetics the unrecognized confounding factor in bioelectromagnetics? Flock-dependence of field-induced anoxia protection in chick embryos. *Bioelectrochem Bioenerg.* 48(1):209-215, 1999.

Diem E, Schwarz C, Adlkofer F, Jahn O, Rudiger H. Non-thermal DNA breakage by mobile-phone radiation (1800MHz) in human fibroblasts and in transformed GFSH-R17 rat granulosa cells in vitro. *Mutat Res.* 583:178-183, 2005.

Ferreira AR, Knakievicz T, de Bittencourt Pasquali MA, Gelain DP, Dal-Pizzol F, Fernandez CE, de Almeida de Salles AA, Ferreira HB, Moreira JC. Ultra high frequency-electromagnetic field irradiation during pregnancy leads to an increase in erythrocytes micronuclei incidence in rat offspring. *Life Sci.* 80:43-50, 2006.

Fucic A, Garaj-Vrhovac V, Skara M, Dimitrovic B, X-rays, microwaves and vinyl chloride monomer: their clastogenic and aneugenic activity, using the micronucleus assay on human lymphocytes. *Mutat Res* 282(4):265-271, 1992.

Gadhia PK, Shah T, Mistry A, Pithawala M, Tamakuwala D. A Preliminary Study to Assess Possible Chromosomal Damage Among Users of Digital Mobile Phones. *Electromag Biol Med* 22:149-159, 2003.

Gandhi G, Anita. Genetic damage in mobile phone users: some preliminary findings. *Ind J Hum Genet* 11(2): 99-104, 2005.

Gandhi G, Singh P. Cytogenetic damage in mobile phone users: preliminary data. *Int J Hum Genet* 5(4):259-265, 2005.

Garaj-Vrhovac V, Horvat D, Koren Z, The effect of microwave radiation on the cell genome. *Mutat Res* 243(2):87-93, 1990.

Garaj-Vrhovac V, Horvat D, Koren Z, The relationship between colony-forming ability, chromosome aberrations and incidence of micronuclei in V79 Chinese hamster cells exposed to microwave radiation. *Mutat Res* 263(3):143-149, 1991.

Garaj-Vrhovac V, Fucic A, Horvat D, The correlation between the frequency of micronuclei and specific chromosome aberrations in human lymphocytes exposed to microwave radiation in vitro. *Mutat Res* 281(3):181-186, 1992.

Garaj-Vrhovac, V, Micronucleus assay and lymphocyte mitotic activity in risk assessment of occupational exposure to microwave radiation. *Chemosphere* 39(13):2301-2312, 1999.

Garson OM, McRobert TL, Campbell LJ, Hocking BA, Gordon I. A chromosomal study of workers with long-term exposure to radio-frequency radiation. *Med J Aust* 155(5):289-292, 1991.

Gos P, Eicher B, Kohli J, Heyer WD. No mutagenic or recombinogenic effects of mobile phone fields at 900 MHz detected in the yeast *saccharomyces cerevisiae*. *Bioelectromagnetics* 21(7):515-523, 2000.

Haider T, Knasmueller S, Kundi M, Haider M. Clastogenic effects of radiofrequency radiations on chromosomes of *Tradescantia*. *Mutat Res* 324(1-2):65-68, 1994.

Hook GJ, Zhang P, Lagroye I, Li L, Higashikubo R, Moros EG, Straube WL, Pickard WF, Baty JD, Roti Roti JL. Measurement of DNA damage and apoptosis in molt-4 cells after in vitro exposure to radiofrequency radiation. *Radiat Res*. 161(2): 193-200, 2004.

Höytö A, Juutilainen J, Naarala J. Ornithine decarboxylase activity is affected in primary astrocytes but not in secondary cell lines exposed to 872 MHz RF radiation. *Int J Radiat Biol*. 83(6):367-374, 2007.

Huss A, Egger M, Hug K, Huwiler-Müntener K, Rösli M. Source of funding and results of studies of health effects of mobile phone use: systematic review of experimental studies. *Environ Health Perspect*. 115(1):1-4, 2007.

Juutilainen J, Heikkinen P, Soikkeli H, Mäki-Paakkanen J. Micronucleus frequency in erythrocytes of mice after long-term exposure to radiofrequency radiation. *Int J Radiat Biol*. 83(4):213-220, 2007.

Kerbacher JJ, Meltz ML, Erwin DN. Influence of radiofrequency radiation on chromosome aberrations in CHO cells and its interaction with DNA-damaging agents. *Radiat Res* 123(3):311-319, 1990.

Komatsubara Y, Hirose H, Sakurai T, Koyama S, Suzuki Y, Taki M, Miyakoshi J. Effect of high-frequency electromagnetic fields with a wide range of SARs on chromosomal aberrations in murine m5S cells. *Mutat Res*. 587(1-2):114-119, 2005.

Koyama S, Isozumi Y, Suzuki Y, Taki M, Miyakoshi J. Effects of 2.45-GHz electromagnetic fields with a wide range of SARs on micronucleus formation in CHO-K1 cells. *ScientificWorldJournal* 4 Suppl 2:29-40, 2004.

Lagroye I, Anane R, Wettring BA, Moros EG, Straube WL, Laregina M, Niehoff M, Pickard WF, Baty J, Roti JL. Measurement of DNA damage after acute exposure to pulsed-wave 2450 MHz microwaves in rat brain cells by two alkaline comet assay methods. *Int J Radiat Biol*. 80(1):11-20, 2004a.

Lagroye I, Hook GJ, Wettring BA, Baty JD, Moros EG, Straube WL, Roti Roti JL. Measurements of Alkali-Labile DNA Damage and Protein-DNA Crosslinks after 2450

MHz Microwave and Low-Dose Gamma Irradiation In Vitro. *Radiat Res.* 161(2): 201-214, 2004b.

Lai H, Singh NP, Acute low-intensity microwave exposure increases DNA single-strand breaks in rat brain cells. *Bioelectromagnetics* 16(3):207-210, 1995.

Lai H, Singh NP, Single- and double-strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radiation. *Int J Radiat Biol* 69(4):513-521, 1996.

Lai, H, Singh, NP, Melatonin and a spin-trap compound block radiofrequency electromagnetic radiation-induced DNA strand breaks in rat brain cells. *Bioelectromagnetics* 18(6):446-454, 1997a.

Lai H, Singh NP. Melatonin and N-tert-butyl-alpha-phenylnitrone block 60-Hz magnetic field-induced DNA single and double strand breaks in rat brain cells. *J Pineal Res.* 22(3):152-162, 1997b.

Lai H, Carino MA, Singh NP, Naltrexone blocks RFR-induced DNA double strand breaks in rat brain cells. *Wireless Networks* 3:471-476, 1997.

Lai H, Singh NP Magnetic-field-induced DNA strand breaks in brain cells of the rat. *Environ Health Perspect.* 112(6):687-694, 2004.

Lai H, Singh NP, Interaction of microwaves and a temporally incoherent magnetic field on single and double DNA strand breaks in rat brain cells. *Electromag Biol Med* 24:23-29, 2005.

Li L, Bisht KS, LaGroye I, Zhang P, Straube WL, Moros EG, Roti Roti JL. Measurement of DNA damage in mammalian cells exposed in vitro to radiofrequency fields at sars of 3-5 w/kg. *Radiat Res* 156:328-332, 2001.

Lixia S, Yao K, Kaijun W, Deqiang L, Huajun H, Xiangwei G, Baohong W, Wei Z, Jianling L, Wei W. Effects of 1.8GHz radiofrequency field on DNA damage and expression of heat shock protein 70 in human lens epithelial cells. *Mutat Res.* 602:135-142, 2006.

Maes A, Verschaeve L, Arroyo A, De Wagter C, Vercruyssen L, In vitro cytogenetic effects of 2450 MHz waves on human peripheral blood lymphocytes. *Bioelectromagnetics* 14(6):495-501, 1993.

Maes A, Collier M, Slaets D, Verschaeve L, 954 MHz microwaves enhance the mutagenic properties of mitomycin C. *Environ Mol Mutagen* 28(1):26-30, 1996.

Maes A, Collier M, Van Gorp U, Vandoninck S, Verschaeve L, Cytogenetic effects of 935.2-MHz (GSM) microwaves alone and in combination with mitomycin C. *Mutat Res* 393(1-2):151-156, 1997.

Maes A, Collier M, Verschaeve L Cytogenetic investigations on microwaves emitted by a 455.7 MHz car phone. *Folia Biol (Praha)* 46(5):175-180, 2000.

Maes A, Collier M, Verschaeve L Cytogenetic effects of 900 MHz (GSM) microwaves on human lymphocytes. *Bioelectromagnetics* 22(2):91-96, 2001.

Maes A, Van Gorp U, Verschaeve L. Cytogenetic investigation of subjects professionally exposed to radiofrequency radiation. *Mutagenesis*. 21:139-142, 2006.

Malyapa RS, Ahern EW, Straube WL, Moros EG, Pickard WF, Roti Roti JL, Measurement of DNA damage after exposure to 2450 MHz electromagnetic radiation. *Radiat Res* 148(6):608-617, 1997a.

Malyapa RS, Ahern EW, Straube WL, Moros EG, Pickard WF, Roti Roti JL, Measurement of DNA damage after exposure to electromagnetic radiation in the cellular phone communication frequency band (835.62 and 847.74 MHz). *Radiat Res* 148(6):618-627, 1997b.

Malyapa RS, Ahern EW, Bi C, Straube WL, LaRegina M, Pickard WF, Roti Roti JL, DNA damage in rat brain cells after in vivo exposure to 2450 MHz electromagnetic radiation and various methods of euthanasia. *Radiat Res* 149(6):637-645, 1998.

Malyapa RS, Bi C, Ahern EW, Roti Roti JL Detection of DNA damage by the alkaline comet assay after exposure to low-dose gamma radiation. *Radiat Res*. 149(4):396-400, 1998.

Markova E, Hillert L, Malmgren L, Persson BR, Belyaev IY. Microwaves from GSM Mobile Telephones Affect 53BP1 and gamma-H2AX Foci in Human Lymphocytes from Hypersensitive and Healthy Persons. *Environ Health Perspect*. 113(9):1172-1177, 2005.

Mashevich M, Folkman D, Kesar A, Barbul A, Korenstein R, Jerby E, Avivi L. Exposure of human peripheral blood lymphocytes to electromagnetic fields associated with cellular phones leads to chromosomal instability. *Bioelectromagnetics* 24:82-90, 2003.

McNamee JP, Bellier PV, Gajda GB, Miller SM, Lemay EP, Lavallee BF, Marro L, Thansandote A. DNA Damage and Micronucleus Induction in Human Leukocytes after Acute In Vitro Exposure to a 1.9 GHz Continuous-Wave Radiofrequency Field. *Radiat Res* 158(4):523-533, 2002a.

McNamee JP, Bellier PV, Gajda GB, Lavallee BF, Lemay EP, Marro L, Thansandote A. DNA Damage in Human Leukocytes after Acute In Vitro Exposure to a 1.9 GHz Pulse-Modulated Radiofrequency Field. *Radiat Res* 158(4):534-537, 2002b.

McNamee JP, Bellier PV, Gajda GB, Lavallee BF, Marro L, Lemay E, Thansandote A. No Evidence for Genotoxic Effects from 24 h Exposure of Human Leukocytes to 1.9 GHz Radiofrequency Fields. *Radiat Res* 159(5):693-697, 2003.

Meltz ML, Eagan P, Erwin DN, Proflavin and microwave radiation: absence of a mutagenic interaction. *Bioelectromagnetics* 11(2):149-157, 1990.

Narasimhan V, Huh WK, Altered restriction patterns of microwave irradiated lambdaphage DNA. *Biochem Int* 25(2):363-370, 1991.

Nikolova T, Czyz J, Rolletschek A, Blyszczuk P, Fuchs J, Jovtchev G, Schuderer J, Kuster N, Wobus AM. Electromagnetic fields affect transcript levels of apoptosis-related genes in embryonic stem cell-derived neural progenitor cells. *ASEB J.* 19(12):1686-1688, 2005.

Ono T, Saito Y, Komura J, Ikehata H, Tarusawa Y, Nojima T, Goukon K, Ohba Y, Wang J, Fujiwara O, Sato R. Absence of mutagenic effects of 2.45 GHz radiofrequency exposure in spleen, liver, brain, and testis of lacZ-transgenic mouse exposed in utero. *Tohoku J Exp Med.* 202(2):93-103, 2004.

Oral B, Guney M, Ozguner F, Karahan N, Mungan T, Comlekci S, Cesur G. Endometrial apoptosis induced by a 900-MHz mobile phone: preventive effects of vitamins E and C. *Adv Ther.* 23(6):957-973, 2006

Paulraj R, Behari J. Single strand DNA breaks in rat brain cells exposed to microwave radiation. *Mutat Res.* 596:76-80, 2006.

Phillips, J.L., Ivaschuk, O., Ishida-Jones, T., Jones, R.A., Campbell-Beachler, M. and Haggren, W. DNA damage in Molt-4 T- lymphoblastoid cells exposed to cellular telephone radiofrequency fields in vitro. *Bioelectrochem. Bioenerg.* 45:103-110, 1998.

Port M, Abend M, Romer B, Van Beuningen D. Influence of high-frequency electromagnetic fields on different modes of cell death and gene expression. *Int J Radiat Biol.* 79(9):701-708, 2003.

Roti Roti JL, Malyapa RS, Bisht KS, Ahern EW, Moros EG, Pickard WF, Straube WL, Neoplastic Transformation in C3H 10T(1/2) Cells after Exposure to 835.62 MHz FDMA and 847.74 MHz CDMA Radiations. *Radiat Res* 155(1):239-247, 2001.

Sakuma N, Komatsubara Y, Takeda H, Hirose H, Sekijima M, Nojima T, Miyakoshi J. DNA strand breaks are not induced in human cells exposed to 2.1425 GHz band CW and W-CDMA modulated radiofrequency fields allocated to mobile radio base stations. *Bioelectromagnetics.* 27:51-57, 2006.

Sarimov R, Malmgren L.O.G., Markova, E., Persson, B.R.R., Belyaev, I.Y. Nonthermal GSM microwaves affect chromatin conformation in human lymphocytes similar to heat shock. *IEEE Trans Plasma Sci* 32:1600-1608, 2004.

Sarkar S, Ali S, Behari J, Effect of low power microwave on the mouse genome: a direct DNA analysis. *Mutat Res* 320(1-2):141-147, 1994.

Scarfi MR, Fresegna AM, Villani P, Pinto R, Marino C, Sarti M, Altavista P, Sannino A, Lovisolo GA. Exposure to radiofrequency radiation (900 MHz, GSM signal) does not affect micronucleus frequency and cell proliferation in human peripheral blood lymphocytes: an interlaboratory study. *Radiat Res.* 165(6):655-663, 2006.

Semin IuA, Shvartsburg LK, Dubovik BV. [Changes in the secondary structure of DNA under the influence of external low-intensity electromagnetic field] *Radiats Biol Radioecol* 35(1):36-41, 1995.

Simkó M Cell type specific redox status is responsible for diverse electromagnetic field effects. *Curr Med Chem.* 14(10):1141-1152, 2007.

Stronati L, Testa A, Moquet J, Edwards A, Cordelli E, Villani P, Marino C, Fresegna AM, Appolloni M, Lloyd D. 935 MHz cellular phone radiation. An in vitro study of genotoxicity in human lymphocytes. *Int J Radiat Biol.* 82(5):339-346, 2006.

Sun LX, Yao K, He JL, Lu DQ, Wang KJ, Li HW. [Effect of acute exposure to microwave from mobile phone on DNA damage and repair of cultured human lens epithelial cells in vitro.] *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi.* 24(8):465-467, 2006.

Sykes PJ, McCallum BD, Bangay MJ, Hooker AM, Morley AA. Effect of Exposure to 900 MHz Radiofrequency Radiation on Intrachromosomal Recombination in pKZ1 Mice. *Radiat Res* 156(5):495-502, 2001.

Takahashi S, Inaguma S, Cho Y-M, Imaida K, Wang J, Fujiwara O, Shirai T, Lack of Mutation Induction with Exposure to 1.5 GHz Electromagnetic Near Fields Used for Cellular Phones in Brains of Big Blue Mice. *Cancer Res* 62:1956-1960, 2002.

Tice RR, Hook GG, Donner M, McRee DI, Guy AW. Genotoxicity of radiofrequency signals. I. Investigation of DNA damage and micronuclei induction in cultured human blood cells. *Bioelectromagnetics* 23:113-126, 2002.

Trosic I. Multinucleated giant cell appearance after whole body microwave irradiation of rats. *Int J Hyg Environ Health.* 204(2-3):133-138, 2001.

Trosic I, Busljeta I, Kasuba V, Rozgaj R. Micronucleus induction after whole-body microwave irradiation of rats. *Mutat Res* 521(1-2):73-79, 2002.

Trosic I, Busljeta I, Modlic B. Investigation of the genotoxic effect of microwave irradiation in rat bone marrow cells: in vivo exposure. *Mutagenesis.* 19(5):361-364, 2004.

Trosic I, Busljeta I. Erythropoietic dynamic equilibrium in rats maintained after microwave irradiation. *Exp Toxicol Pathol.* 57(3):247-251, 2006.

Verschaeve, L., Heikkinen, P., Verheyen, G., Van Gorp, U., Boonen, F., Vander Plaetse, F., Maes, A., Kumlin, T., Maki-Paakkanen, J., Puranen, L. and Juutilainen, J.

Investigation of Co-genotoxic Effects of Radiofrequency Electromagnetic Fields In Vivo. *Radiat. Res.* 165, 598-607, 2006.

Vijayalaxmi, Mohan, N, Meltz, ML, Wittler, MA, Proliferation and cytogenetic studies in human blood lymphocytes exposed in vitro to 2450 MHz radiofrequency radiation. *Int J Radiat Biol* 72(6):751-757, 1997a.

Vijayalaxmi, Frei, MR, Dusch, SJ, Guel, V, Meltz, ML, Jauchem, JR, Frequency of micronuclei in the peripheral blood and bone marrow of cancer-prone mice chronically exposed to 2450 MHz radiofrequency radiation. *Radiat Res* 147(4):495-500, 1997b.

Vijayalaxmi, Seaman RL, Belt ML, Doyle JM, Mathur SP, Prihoda TJ., Frequency of micronuclei in the blood and bone marrow cells of mice exposed to ultra-wideband electromagnetic radiation. *Int J Radiat Biol.* 75(1):115-120, 1999.

Vijayalaxmi, Leal BZ, Szilagyi M, Prihoda TJ, Meltz ML, Primary DNA Damage in Human Blood Lymphocytes Exposed In Vitro to 2450 MHz Radiofrequency Radiation. *Radiat Res* 153(4):479-486, 2000.

Vijayalaxmi, Pickard WF, Bisht KS, Prihoda TJ, Meltz ML, LaRegina MC, Roti Roti JL, Straube WL, Moros EG. Micronuclei in the peripheral blood and bone marrow cells of rats exposed to 2450 MHz radiofrequency

Vijayalaxmi, Leal BZ, Meltz ML, Pickard WF, Bisht KS, Roti Roti JL, Straube WL, Moros EG, Cytogenetic Studies in Human Blood Lymphocytes Exposed In Vitro to Radiofrequency Radiation at a Cellular Telephone Frequency (835.62 MHz, FDMA). *Radiat Res* 155(1):113-121, 2001b.

Vijayalaxmi, Bisht KS, Pickard WF, Meltz ML, Roti Roti JL, Moros EG. Chromosome damage and micronucleus formation in human blood lymphocytes exposed in vitro to radiofrequency radiation at a cellular telephone frequency (847.74 MHz, CDMA). *Radiat Res* 156(4):430-432, 2001c.

Vijayalaxmi, Sasser LB, Morris JE, Wilson BW, Anderson LE. Genotoxic Potential of 1.6 GHz Wireless Communication Signal: In Vivo Two-Year Bioassay. *Radiat Res* 159(4):558-564, 2003.

Zeni, O., Schiavoni, A. S., Sannino, A., Antolini, A., Forigo, D., Bersani, F. and Scarfi, M. R. Lack of Genotoxic Effects (Micronucleus Induction) in Human Lymphocytes Exposed In Vitro to 900 MHz Electromagnetic Fields. *Radiat. Res.* 160, 152-158, 2003.

Zeni O, Romano M, Perrotta A, Lioi MB, Barbieri R, d'Ambrosio G, Massa R, Scarfi MR. Evaluation of genotoxic effects in human peripheral blood leukocytes following an acute in vitro exposure to 900 MHz radiofrequency fields. *Bioelectromagnetics.* 26(4):258-265, 2005.

Zhang DY, Xu ZP, Chiang H, Lu DQ, Zeng QL. [Effects of GSM 1800 MHz

radiofrequency electromagnetic fields on DNA damage in Chinese hamster lung cells.] *Zhonghua Yu Fang Yi Xue Za Zhi.* 40(3):149-152, 2006.

Zhang MB, He JL, Jin LF, Lu DQ. Study of low-intensity 2450-MHz microwave exposure enhancing the genotoxic effects of mitomycin C using micronucleus test and comet assay in vitro. *Biomed Environ Sci* 15(4):283-290, 2002.

Zotti-Martelli L, Peccatori M, Scarpato R, Migliore L, Induction of micronuclei in human lymphocytes exposed in vitro to microwave radiation. *Mutat Res* 472(1-2):51-58, 2000.

Zotti-Martelli L, Peccatori M, Maggini V, Ballardini M, Barale R. Individual responsiveness to induction of micronuclei in human lymphocytes after exposure in vitro to 1800-MHz microwave radiation. *Mutat Res.* 582(1-2):42-52, 2005.

APPENDIX 6-A**Abstracts on Effects of Extremely Low Frequency (ELF) EMF on DNA**

27 (E)- effect reported; 14 (NE)- no significant effect reported

Ahuja YR, Vijayashree B, Saran R, Jayashri EL, Manoranjani JK, Bhargava SC. In vitro effects of low-level, low-frequency electromagnetic fields on DNA damage in human leucocytes by comet assay. Indian J Biochem Biophys. 36(5):318-322, 1999. (E)

The sources for the effects of electromagnetic fields (EMFs) have been traced to time-varying as well as steady electric and magnetic fields, both at low and high to ultra high frequencies. Of these, the effects of low-frequency (50/60 HZ) magnetic fields, directly related to time-varying currents, are of particular interest as exposure to some fields may be commonly experienced. In the present study, investigations have been carried out at low-level (mT) and low-frequency (50 Hz) electromagnetic fields in healthy human volunteers. Their peripheral blood samples were exposed to 5 doses of electromagnetic fields (2,3,5,7 and 10mT at 50 Hz) and analysed by comet assay. The results were compared to those obtained from unexposed samples from the same subjects. 50 cells per treatment per individual were scored for comet-tail length which is an estimate of DNA damage. Data from observations among males were pooled for each flux density for analysis. At each flux density, with one exception, there was a significant increase in the DNA damage from the control value. When compared with a similar study on females carried out by us earlier, the DNA damage level was significantly higher in the females as compared to the males for each flux density.

Cantoni O, Sestili P, Fiorani M, Dacha M. Effect of 50 Hz sinusoidal electric and/or magnetic fields on the rate of repair of DNA single strand breaks in cultured mammalian cells exposed to three different carcinogens: methylmethane sulphonate, chromate and 254 nm U.V. radiation. Biochem Mol Biol Int. 38(3):527-533, 1996. (NE)

Treatment of cultured mammalian cells with three different carcinogens, namely methylmethane sulphonate (MMS), chromate and 254 U.V. radiation, produces DNA single strand breaks (SSB) in cultured mammalian cells. The rate of removal of these lesions is not affected by exposure to 50 Hz electric (0.2 - 20 kV/m), magnetic (0.0002-0.2 mT), or combined electric and magnetic fields. These results indicate that, under the experimental conditions utilized in this study, 50 Hz electric, magnetic and electromagnetic fields (over a wide range of intensities) do not affect the machinery involved in the repair of DNA SSBs generated by different carcinogens in three different cultured mammalian cell lines, making it unlikely that field exposure enhances the ability of these carcinogens to induce transformation via inhibition of DNA repair.

Chahal R, Craig DQ, Pinney RJ. Investigation of potential genotoxic effects of low frequency electromagnetic fields on Escherichia coli. J Pharm Pharmacol. 45(1):30-33, 1993. (NE)

Exposure of growing cells of Escherichia coli strain AB1157 to a frequency of 1 Hz with field strengths of 1 or 3 kV m⁻¹ did not affect spontaneous or ultraviolet light (UV)-induced mutation frequencies to rifampicin resistance. Neither did growth in the presence of charge alter the sensitivities of strains AB1157, TK702 umuC or TK501 umuC uvrB to UV. Similarly, although the resistance of strains TK702 umuC and TK501 umuC uvrB to UV was increased by the presence of plasmid pKM101, which carries DNA repair genes, pregrowth of plasmid-containing strains in electric fields did not increase UV resistance. Finally, growth in a low frequency field in the presence of sub-inhibitory concentrations of mitomycin C did not affect mitomycin C-induced mutation frequencies. It is concluded that low frequency electromagnetic fields do not increase spontaneous mutation, induce DNA repair or increase the mutagenic effects of UV or mitomycin C.

Chow K, Tung WL Magnetic field exposure enhances DNA repair through the induction of DnaK/J synthesis. FEBS Lett. 478(1-2):133-136, 2000. (E)

In contrast to the common impression that exposure to a magnetic field of low frequency causes mutations to organisms, we have demonstrated that a magnetic field can actually enhance the efficiency of DNA repair. Using Escherichia coli strain XL-1 Blue as the host and plasmid pUC8 that had been mutagenized by hydroxylamine as the vector for assessment, we found that bacterial transformants that had been exposed to a magnetic field of 50 Hz gave lower percentages of white colonies as compared to transformants that had not been exposed to the magnetic field. This result was indicative that the efficiency of DNA repair had been improved. The improvement was found to be mediated by the induced overproduction of heat shock proteins DnaK/J (Hsp70/40).

Delimaris J, Tsilimigaki S, Messini-Nicolaki N, Ziros E, Piperakis SM Effects of pulsed electric fields on DNA of human lymphocytes. Cell Biol Toxicol. 22(6):409-415, 2006. (E)

The effects of pulsed electric fields of low frequency (50 Hz) on DNA of human lymphocytes were investigated. The influence of additional external factors, such as hydrogen peroxide (H₂O₂) and gamma-irradiation, as well as the repair efficiency in these lymphocytes, was also evaluated. The comet assay, a very sensitive and rapid method for detecting DNA damage at the single cells level was the method used. A significant amount of damage was observed after exposure to the electric fields, compared to the controls. After 2 h incubation at 37 degrees C, a proportion of damage was repaired. H₂O₂ and gamma-irradiation increased the damage to lymphocytes exposed to pulsed electric fields according to the dose used, while the amount of the repair was proportional to the damage.

Fairbairn DW, O'Neill KL The effect of electromagnetic field exposure on the formation of DNA single strand breaks in human cells. Cell Mol Biol (Noisy-le-grand). 40(4):561-567, 1994. (NE)

Electromagnetic fields (EMF) have been reported to be associated with human cancers in a number of epidemiological studies. Agents that are associated with cancer affect DNA in an adverse manner. This is a report of a DNA damage study in human cells exposed to EMFs. Single strand breaks in DNA are proposed to be necessary events in both mutagenesis and carcinogenesis. The single cell gel assay is a sensitive and accurate technique that was used in this study for single strand break detection. The EMF exposure system used here appeared to have no direct effect on DNA damage induction in a series of experiments. Moreover, EMF did not have a significant effect in potentiating DNA damage in cells treated with oxidative stresses.

Fiorani M, Cantoni O, Sestili P, Conti R, Nicolini P, Vetrano F, Dacha M. Electric and/or magnetic field effects on DNA structure and function in cultured human cells. *Mutat Res.* 282(1):25-29, 1992. (NE)

Exposure of cultured K562 cells to 50 Hz electric (0.2-20 kV/m), magnetic (0.002-2 G), or combined electric and magnetic fields for up to 24 h did not result in the production of detectable DNA lesions, as assayed by the filter elution technique. The rate of cell growth was also unaffected as well as the intracellular ATP and NAD⁺ levels. These results indicate that, under the experimental conditions utilized in this study, 50 Hz electric, magnetic and electromagnetic fields are not geno- and cyto-toxic in cultured mammalian cells.

Frazier ME, Reese JA, Morris JE, Jostes RF, Miller DL Exposure of mammalian cells to 60-Hz magnetic or electric fields: analysis of DNA repair of induced, single-strand breaks. *Bioelectromagnetics.* 11(3):229-234, 1990. (NE)

DNA damage was induced in isolated human peripheral lymphocytes by exposure at 5 Gy to 60Co radiation. Cells were permitted to repair the DNA damage while exposed to 60-Hz fields or while sham-exposed. Exposed cells were subjected to magnetic (B) or electric (E) fields, alone or in combination, throughout their allotted repair time. Repair was stopped at specific times, and the cells were immediately lysed and then analyzed for the presence of DNA single-strand breaks (SSB) by the alkaline-elution technique. Fifty to 75 percent of the induced SSB were repaired 20 min after exposure, and most of the remaining damage was repaired after 180 min. Cells were exposed to a 60-Hz ac B field of 1 mT; an E field of 1 or 20 V/m; or combined E and B fields of 0.2 V/m and 0.05 mT, 6 V/m and 0.6 mT, or 20 V/m and 1 mT. None of the exposures was observed to affect significantly the repair of DNA SSB.

Hong R, Zhang Y, Liu Y, Weng EQ. [Effects of extremely low frequency electromagnetic fields on DNA of testicular cells and sperm chromatin structure in mice] *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi.* 23(6):414-417, 2005. (E)

[Article in Chinese]

OBJECTIVE: To study the effects of 50 Hz electromagnetic fields (EMFs) on DNA of testicular cells and sperm chromatin structure in mice. **METHODS:** Mice were exposed to 50 Hz, 0.2 mT or 6.4 mT electromagnetic fields for 4 weeks. DNA strand breakage in testicular cells was detected by single-cell gel electrophoresis assay. Sperm chromatin structure was analyzed by sperm chromatin structure assay with flow cytometry. **RESULTS:** After 50 Hz, 0.2 mT or 6.4 mT EMFs exposure, the percentage of cells with DNA migration in total testicular cells increased from the control level of 25.64% to 37.83% and 39.38% respectively. The relative length of comet tail and the percentage of DNA in comet tail respectively increased from the control levels of 13.06% \pm 12.38% and 1.52% \pm 3.25% to 17.86% \pm 14.60% and 2.32% \pm 4.26% after 0.2 mT exposure and to 17.88% \pm 13.71% and 2.35% \pm 3.87% after 6.4 mT exposure ($P < 0.05$). Exposure to EMFs had not induced significant changes in S.D.alphaT and XalphaT, but COMPalphaT (cells outside the main population of alpha t), the percentage of sperms with abnormal chromatin structure, increased in the two exposed groups. **CONCLUSION:** 50 Hz EMFs may have the potential to induce DNA strand breakage in testicular cells and sperm chromatin condensation in mice.

Ivancsits S, Pilger A, Diem E, Jahn O, Rudiger HW. Cell type-specific genotoxic effects of intermittent extremely low-frequency electromagnetic fields. *Mutat Res.* 583(2):184-188, 2005. (E)

The issue of adverse health effects of extremely low-frequency electromagnetic fields (ELF-EMFs) is highly controversial. Contradictory results regarding the genotoxic potential of ELF-EMF have been reported in the literature. To test whether this controversy might reflect differences between the cellular targets examined we exposed cultured cells derived from different tissues to an intermittent ELF-EMF (50 Hz sinusoidal, 1 mT) for 1-24h. The alkaline and neutral comet assays were used to assess ELF-EMF-induced DNA strand breaks. We could identify three responder (human fibroblasts, human melanocytes, rat granulosa cells) and three non-responder cell types (human lymphocytes, human monocytes, human skeletal muscle cells), which points to the significance of the cell system used when investigating genotoxic effects of ELF-EMF.

Ivancsits S, Diem E, Jahn O, Rudiger HW. Age-related effects on induction of DNA strand breaks by intermittent exposure to electromagnetic fields. *Mech Ageing Dev.* 124(7):847-850, 2003. (E)

Several studies indicating a decline of DNA repair efficiency with age raise the question, if senescence per se leads to a higher susceptibility to DNA damage upon environmental exposures. Cultured fibroblasts of six healthy donors of different age exposed to intermittent ELF-EMF (50 Hz sinus, 1 mT) for 1-24 h exhibited different basal DNA strand break levels correlating with age. The cells revealed a maximum response at 15-19 h of exposure. This response was clearly more pronounced in cells from older donors, which could point to an age-related decrease of DNA repair efficiency of ELF-EMF induced DNA strand breaks.

Ivancsits S, Diem E, Pilger A, Rudiger HW, Jahn O. Induction of DNA strand breaks by intermittent exposure to extremely-low-frequency electromagnetic fields in human diploid fibroblasts. Mutat Res. 519(1-2):1-13, 2002. (E)

Results of epidemiological research show low association of electromagnetic field (EMF) with increased risk of cancerous diseases and missing dose-effect relations. An important component in assessing potential cancer risk is knowledge concerning any genotoxic effects of extremely-low-frequency-EMF (ELF-EMF). Human diploid fibroblasts were exposed to continuous or intermittent ELF-EMF (50Hz, sinusoidal, 24h, 1000microT). For evaluation of genotoxic effects in form of DNA single- (SSB) and double-strand breaks (DSB), the alkaline and the neutral comet assay were used. In contrast to continuous ELF-EMF exposure, the application of intermittent fields reproducibly resulted in a significant increase of DNA strand break levels, mainly DSBs, as compared to non-exposed controls. The conditions of intermittence showed an impact on the induction of DNA strand breaks, producing the highest levels at 5min field-on/10min field-off. We also found individual differences in response to ELF-EMF as well as an evident exposure-response relationship between magnetic flux density and DNA migration in the comet assay. Our data strongly indicate a genotoxic potential of intermittent EMF. This points to the need of further studies in vivo and consideration about environmental threshold values for ELF exposure.

Ivancsits S, Diem E, Pilger A, Rudiger HW, Jahn O. Induction of DNA strand breaks by intermittent exposure to extremely-low-frequency electromagnetic fields in human diploid fibroblasts. Mutat Res. 519(1-2):1-13, 2002. (E)

Results of epidemiological research show low association of electromagnetic field (EMF) with increased risk of cancerous diseases and missing dose-effect relations. An important component in assessing potential cancer risk is knowledge concerning any genotoxic effects of extremely-low-frequency-EMF (ELF-EMF). Human diploid fibroblasts were exposed to continuous or intermittent ELF-EMF (50Hz, sinusoidal, 24h, 1000microT). For evaluation of genotoxic effects in form of DNA single- (SSB) and double-strand breaks (DSB), the alkaline and the neutral comet assay were used. In contrast to continuous ELF-EMF exposure, the application of intermittent fields reproducibly resulted in a significant increase of DNA strand break levels, mainly DSBs, as compared to non-exposed controls. The conditions of intermittence showed an impact on the induction of DNA strand breaks, producing the highest levels at 5min field-on/10min field-off. We also found individual differences in response to ELF-EMF as well as an evident exposure-response relationship between magnetic flux density and DNA migration in the comet assay. Our data strongly indicate a genotoxic potential of intermittent EMF. This points to the need of further studies in vivo and consideration about environmental threshold values for ELF exposure.

Jajte J, Zmyslony M, Palus J, Dziubaltowska E, Rajkowska E. Protective effect of

melatonin against in vitro iron ions and 7 mT 50 Hz magnetic field-induced DNA damage in rat lymphocytes. *Mutat Res.* 483(1-2):57-64, 2001. (E)

We have previously shown that simultaneous exposure of rat lymphocytes to iron ions and 50Hz magnetic field (MF) caused an increase in the number of cells with DNA strand breaks. Although the mechanism of MF-induced DNA damage is not known, we suppose that it involves free radicals. In the present study, to confirm our hypothesis, we have examined the effect of melatonin, an established free radicals scavenger, on DNA damage in rat peripheral blood lymphocytes exposed in vitro to iron ions and 50Hz MF. The alkaline comet assay was chosen for the assessment of DNA damage. During pre-incubation, part of the cell samples were supplemented with melatonin (0.5 or 1.0mM). The experiments were performed on the cell samples incubated for 3h in Helmholtz coils at 7mT 50Hz MF. During MF exposure, some samples were treated with ferrous chloride (FeCl₂, 10microg/ml), while the rest served as controls. A significant increase in the number of cells with DNA damage was found only after simultaneous exposure of lymphocytes to FeCl₂ and 7mT 50Hz MF, compared to the control samples or those incubated with FeCl₂ alone. However, when the cells were treated with melatonin and then exposed to iron ions and 50Hz MF, the number of damaged cells was significantly reduced, and the effect depended on the concentration of melatonin. The reduction reached about 50% at 0.5mM and about 100% at 1.0mM. Our results indicate that melatonin provides protection against DNA damage in rat lymphocytes exposed in vitro to iron ions and 50Hz MF (7mT). Therefore, it can be suggested that free radicals may be involved in 50Hz magnetic field and iron ions-induced DNA damage in rat blood lymphocytes. The future experimental studies, in vitro and in vivo, should provide an answer to the question concerning the role of melatonin in the free radical processes in the power frequency magnetic field.

Kindzelskii AL, Petty HR. Extremely low frequency pulsed DC electric fields promote neutrophil extension, metabolic resonance and DNA damage when phase-matched with metabolic oscillators. *Biochim Biophys Acta.* 1495(1):90-111, 2000. (E)

Application of extremely low frequency pulsed DC electric fields that are frequency- and phase-matched with endogenous metabolic oscillations leads to greatly exaggerated neutrophil extension and metabolic resonance wherein oscillatory NAD(P)H amplitudes are increased. In the presence of a resonant field, migrating cell length grows from 10 to approximately 40 microm, as does the overall length of microfilament assemblies. In contrast, cells stop locomotion and become spherical when exposed to phase-mismatched fields. Although cellular effects were not found to be dependent on electrode type and buffer, they were sensitive to temporal constraints (phase and pulse length) and cell surface charge. We suggest an electromechanical coupling hypothesis wherein applied electric fields and cytoskeletal polymerization forces act together to overcome the surface/cortical tension of neutrophils, thus promoting net cytoskeletal assembly and heightened metabolic amplitudes. Metabolic resonance enhances reactive oxygen metabolic production by neutrophils. Furthermore, cellular DNA damage was observed after prolonged metabolic resonance using both single cell gel electrophoresis ('comet' assay) and 3'-OH DNA labeling using terminal deoxynucleotidyl transferase. These

results provide insights into transmembrane signal processing and cell interactions with weak electric fields.

Lai H, Singh NP. Acute exposure to a 60 Hz magnetic field increases DNA strand breaks in rat brain cells. Bioelectromagnetics. 18(2):156-165, 1997. (E)

Acute (2 h) exposure of rats to a 60 Hz magnetic field (flux densities 0.1, 0.25, and 0.5 mT) caused a dose-dependent increase in DNA strand breaks in brain cells of the animals (assayed by a microgel electrophoresis method at 4 h postexposure). An increase in single-strand DNA breaks was observed after exposure to magnetic fields of 0.1, 0.25, and 0.5 mT, whereas an increase in double-strand DNA breaks was observed at 0.25 and 0.5 mT. Because DNA strand breaks may affect cellular functions, lead to carcinogenesis and cell death, and be related to onset of neurodegenerative diseases, our data may have important implications for the possible health effects of exposure to 60 Hz magnetic fields.

Lai H, Singh NP. Magnetic-field-induced DNA strand breaks in brain cells of the rat. Environ Health Perspect. 112(6):687-694, 2004. (E)

In previous research, we found that rats acutely (2 hr) exposed to a 60-Hz sinusoidal magnetic field at intensities of 0.1-0.5 millitesla (mT) showed increases in DNA single- and double-strand breaks in their brain cells. Further research showed that these effects could be blocked by pretreating the rats with the free radical scavengers melatonin and N-tert-butyl-alpha-phenylnitron, suggesting the involvement of free radicals. In the present study, effects of magnetic field exposure on brain cell DNA in the rat were further investigated. Exposure to a 60-Hz magnetic field at 0.01 mT for 24 hr caused a significant increase in DNA single- and double-strand breaks. Prolonging the exposure to 48 hr caused a larger increase. This indicates that the effect is cumulative. In addition, treatment with Trolox (a vitamin E analog) or 7-nitroindazole (a nitric oxide synthase inhibitor) blocked magnetic-field-induced DNA strand breaks. These data further support a role of free radicals on the effects of magnetic fields. Treatment with the iron chelator deferiprone also blocked the effects of magnetic fields on brain cell DNA, suggesting the involvement of iron. Acute magnetic field exposure increased apoptosis and necrosis of brain cells in the rat. We hypothesize that exposure to a 60-Hz magnetic field initiates an iron-mediated process (e.g., the Fenton reaction) that increases free radical formation in brain cells, leading to DNA strand breaks and cell death. This hypothesis could have an important implication for the possible health effects associated with exposure to extremely low-frequency magnetic fields in the public and occupational environments.

Lai H, Singh NP. Melatonin and N-tert-butyl-alpha-phenylnitron block 60-Hz magnetic field-induced DNA single and double strand breaks in rat brain cells. J Pineal Res. 22(3):152-162, 1997. (E)

In previous research, we have found an increase in DNA single- and double-strand breaks in brain cells of rats after acute exposure (two hours) to a sinusoidal 60-Hz magnetic field. The present experiment was carried out to investigate whether treatment with melatonin and the spin-trap compound N-tert-butyl-alpha-phenylnitron (PBN) could

block the effect of magnetic fields on brain cell DNA. Rats were injected with melatonin (1 mg/kg, sc) or PBN (100 mg/kg, ip) immediately before and after two hours of exposure to a 60-Hz magnetic field at an intensity of 0.5 mT. We found that both drug treatments blocked the magnetic field-induced DNA single- and double-strand breaks in brain cells, as assayed by a microgel electrophoresis method. Since melatonin and PBN are efficient free radical scavengers, these data suggest that free radicals may play a role in magnetic field-induced DNA damage.

Li SH, Chow KC. Magnetic field exposure induces DNA degradation. *Biochem Biophys Res Commun.* 280(5):1385-1388, 2001. (E)

In our earlier experiments, we discovered that magnetic field exposure could bring both stabilizing and destabilizing effects to the DNA of *Escherichia coli*, depending on our parameters of assessment, and both of these effects were associated with the induced synthesis of the heat shock proteins Hsp70/Hsp40 (DnaK/DnaJ). These contradicting results prompted us to explore in this study the effect of magnetic field exposure on the DNA stability in vivo when the heat shock response of the cell was suppressed. By using plasmid pUC18 in *E. coli* as the indicator, we found that without the protection of the heat shock response, magnetic field exposure indeed induced DNA degradation and this deleterious effect could be diminished by the presence of an antioxidant, Trolox C. In our in vitro test, we also showed that the magnetic field could potentiate the activity of oxidant radicals.

Lopucki M, Schmerold I, Dadak A, Wiktor H, Niedermuller H, Kankofer M. Low dose magnetic fields do not cause oxidative DNA damage in human placental cotyledons in vitro. *Virchows Arch.* 446(6):634-639, 2005. (NE)

The biological impact of low dose magnetic fields generated by electric appliances present in the human environment is still uncertain. In this study, human placentas served as a model tissue for the evaluation of the potential effect of oscillating low intensity magnetic fields on the concentration of 8-hydroxy-2'-deoxyguanosine (8-OH-dG) in cellular DNA. Cotyledons were dissected from placentas obtained immediately after physiological labours and exposed to magnetic fields (groups MF A, 2 mT, 50 Hz and MF B, 5 mT, 50 Hz) or sham exposed (group C) during an in vitro perfusion of 3 h. Cellular DNA was isolated, hydrolyzed and analyzed by HPLC. Native nucleosides were monitored at 254 nm and 8-OH-dG by electrochemical detection. Results were expressed as μmol 8-OH-dG/mol deoxyguanosine (dG). The concentrations of 8-OH-dG in group C, MF A and MF B were 28.45 ± 15.27 $\mu\text{mol/mol}$ dG, 62.80 ± 31.91 $\mu\text{mol/mol}$ dG, and 27.49 ± 14.23 $\mu\text{mol/mol}$ dG, respectively, demonstrating no significant difference between the groups. The results suggest that placental tissues possess a capacity to protect DNA against oxidative alterations by magnetic field of intensities previously shown to produce radical mediated DNA damage in rat brain cells in vivo and imbalances in electrolyte release of cotyledons under in vitro conditions.

Lourencini da Silva R, Albano F, Lopes dos Santos LR, Tavares AD Jr, Felzenszwalb I. The effect of electromagnetic field exposure on the formation of DNA lesions. Redox Rep. 5(5):299-301, 2000. (E)

In an attempt to determine whether electromagnetic field (EMF) exposure might lead to DNA damage, we exposed SnCl₂-treated pBR322 plasmids to EMF and analysed the resulting conformational changes using agarose gel electrophoresis. An EMF-dependent potentiation of DNA scission (i.e. the appearance of relaxed plasmids) was observed. In confirmation of this, plasmids pre-exposed to EMF also were less capable of transforming *Escherichia coli*. The results indicate that EMF, in the presence of a transition metal, is capable of causing DNA damage. These observations support the idea that EMF, probably through secondary generation of reactive oxygen species, can be clastogenic and provide a possible explanation for the observed correlation between EMF exposure and the frequency of certain types of cancers in humans.

Luceri C, De Filippo C, Giovannelli L, Blangiardo M, Cavalieri D, Aglietti F, Pampaloni M, Andreuccetti D, Pieri L, Bambi F, Biggeri A, Dolara P. Extremely low-frequency electromagnetic fields do not affect DNA damage and gene expression profiles of yeast and human lymphocytes. Radiat Res. 164(3):277-285, 2005. (NE)

We studied the effects of extremely low-frequency (50 Hz) electromagnetic fields (EMFs) on peripheral human blood lymphocytes and DBY747 *Saccharomyces cerevisiae*. Graded exposure to 50 Hz magnetic flux density was obtained with a Helmholtz coil system set at 1, 10 or 100 microT for 18 h. The effects of EMFs on DNA damage were studied with the single-cell gel electrophoresis assay (comet assay) in lymphocytes. Gene expression profiles of EMF-exposed human and yeast cells were evaluated with DNA microarrays containing 13,971 and 6,212 oligonucleotides, respectively. After exposure to the EMF, we did not observe an increase in the amount of strand breaks or oxidated DNA bases relative to controls or a variation in gene expression profiles. The results suggest that extremely low-frequency EMFs do not induce DNA damage or affect gene expression in these two different eukaryotic cell systems.

McNamee JP, Bellier PV, McLean JR, Marro L, Gajda GB, Thansandote A. DNA damage and apoptosis in the immature mouse cerebellum after acute exposure to a 1 mT, 60 Hz magnetic field. Mutat Res. 513(1-2):121-133, 2002. (NE)

Several recent studies have reported that whole-body exposure of rodents to power frequency magnetic fields (MFs) can result in DNA single- and double-strand breaks in the brains of these animals. The current study was undertaken to investigate whether an acute 2h exposure of a 1 mT, 60 Hz MF could elicit DNA damage, and subsequently apoptosis, in the brains of immature (10-day-old) mice. DNA damage was quantitated at 0, 2, 4, and 24h after exposure using the alkaline comet assay. Apoptosis was quantitated in the external granule cell layer (EGCL) of the immature mouse cerebellum at 0 and 24h after exposure to MF by the TdT-mediated dUTP nick-end labeling (TUNEL) assay. Four parameters (tail ratio, tail moment, comet length and tail length) were used to assess DNA damage for each comet. While increased DNA damage was detected by tail ratio at

2h after MF exposure, no supporting evidence of increased DNA damage was detected by the other parameters. In addition, no similar differences were observed using these parameters at any of the other post-exposure times. No increase in apoptosis was observed in the EGCL of MF-exposed mice, when compared to sham mice. Taken together, these results do not support the hypothesis that acute MF exposure causes DNA damage in the cerebellums of immature mice.

McNamee JP, Bellier PV, Chauhan V, Gajda GB, Lemay E, Thansandote A. Evaluating DNA damage in rodent brain after acute 60 Hz magnetic-field exposure. *Radiat Res.* 164(6):791-797, 2005. (NE)

In recent years, numerous studies have reported a weak association between 60 Hz magnetic-field exposure and the incidence of certain cancers. To date, no mechanism to explain these findings has been identified. The objective of the current study was to investigate whether acute magnetic-field exposure could elicit DNA damage within brain cells from both whole brain and cerebellar homogenates from adult rats, adult mice and immature mice. Rodents were exposed to a 60 Hz magnetic field (0, 0.1, 1 or 2 mT) for 2 h. Then, at 0, 2 and 4 h after exposure, animals were killed humanely, their brains were rapidly removed and homogenized, and cells were cast into agarose gels for processing by the alkaline comet assay. Four parameters (tail ratio, tail moment, comet length and tail length) were used to assess DNA damage for each comet. For each species, a significant increase in DNA damage was detected by each of the four parameters in the positive control (2 Gy X rays) relative to the concurrent nonirradiated negative and sham controls. However, none of the four parameters detected a significant increase in DNA damage in brain cell homogenates from any magnetic-field exposure (0- 2 mT) at any time after exposure. The dose-response and time-course data from the multiple animal groups tested in this study provide no evidence of magnetic-field-induced DNA damage.

Miyakoshi J, Yoshida M, Shibuya K, Hiraoka M. Exposure to strong magnetic fields at power frequency potentiates X-ray-induced DNA strand breaks. *J Radiat Res (Tokyo).* 41(3):293-302, 2000. (E)

We examined the effect of an extremely low-frequency magnetic field (ELFMF) at 5, 50 and 400 mT on DNA strand breaks in human glioma MO54 cells. A DNA damage analysis was performed using the method of alkaline comet assay. The cells were exposed to X-rays alone (5 Gy), ELFMF alone, or X-rays followed by ELFMF at 4 degrees C or on ice. No significant difference in the tail moment was observed between control and ELFMF exposures up to 400 mT. X-ray irradiation increased DNA strand breaks. When cells were exposed to X-rays followed by ELFMF at 50 and 400 mT, the tail moment increased significantly compared with that for X-rays alone. When the exposure of cells was performed at 37 degrees C, no significant change was observed between X-rays alone and X-rays plus 400 mT. We previously observed that exposure to 400 mT ELFMF for 2 h increased X-ray-induced mutations (Miyakoshi et al, *Mutat. Res.*, 349: 109-114, 1996). Additionally, an increase in the mutation by exposure to the ELFMF was observed in cells during DNA-synthesizing phase (Miyakoshi et al., *Int. J.*

Radiat. Biol., 71: 75-79, 1997). From these results, it appears that exposure to the high density ELFMF at more than 50 mT may potentiate X-ray-induced DNA strand breaks.

Moretti M, Villarini M, Simonucci S, Fatigoni C, Scassellati-Sforzolini G, Monarca S, Pasquini R, Angelucci M, Strappini M Effects of co-exposure to extremely low frequency (ELF) magnetic fields and benzene or benzene metabolites determined in vitro by the alkaline comet assay. Toxicol Lett. 157(2):119-128, 2005. (E)

In the present study, we investigated in vitro the possible genotoxic and/or co-genotoxic activity of 50 Hz (power frequency) magnetic fields (MF) by using the alkaline single-cell microgel-electrophoresis (comet) assay. Sets of experiments were performed to evaluate the possible interaction between 50 Hz MF and the known leukemogen benzene. Three benzene hydroxylated metabolites were also evaluated: 1,2-benzenediol (1,2-BD, catechol), 1,4-benzenediol (1,4-BD, hydroquinone), and 1,2,4-benzenetriol (1,2,4-BT). MF (1 mT) were generated by a system consisting of a pair of parallel coils in a Helmholtz configuration. To evaluate the genotoxic potential of 50 Hz MF, Jurkat cell cultures were exposed to 1 mT MF or sham-exposed for 1h. To evaluate the co-genotoxic activity of MF, the xenobiotics (benzene, catechol, hydroquinone, and 1,2,4-benzenetriol) were added to Jurkat cells subcultures at the beginning of the exposure time. In cell cultures co-exposed to 1 mT (50 Hz) MF, benzene and catechol did not show any genotoxic activity. However, co-exposure of cell cultures to 1 mT MF and hydroquinone led to the appearance of a clear genotoxic effect. Moreover, co-exposure of cell cultures to 1 mT MF and 1,2,4-benzenetriol led to a marked increase in the genotoxicity of the ultimate metabolite of benzene. The possibility that 50 Hz (power frequency) MF might interfere with the genotoxic activity of xenobiotics has important implications, since human populations are likely to be exposed to a variety of genotoxic agents concomitantly with exposure to this type of physical agent.

Nikolova T, Czyz J, Rolletschek A, Blyszczuk P, Fuchs J, Jovtchev G, Schuderer J, Kuster N, Wobus AM. Electromagnetic fields affect transcript levels of apoptosis-related genes in embryonic stem cell-derived neural progenitor cells. ASEB J. 19(12):1686-1688, 2005. (E)

Mouse embryonic stem (ES) cells were used as an experimental model to study the effects of electromagnetic fields (EMF). ES-derived nestin-positive neural progenitor cells were exposed to extremely low frequency EMF simulating power line magnetic fields at 50 Hz (ELF-EMF) and to radiofrequency EMF simulating the Global System for Mobile Communication (GSM) signals at 1.71 GHz (RF-EMF). Following EMF exposure, cells were analyzed for transcript levels of cell cycle regulatory, apoptosis-related, and neural-specific genes and proteins; changes in proliferation; apoptosis; and cytogenetic effects. Quantitative RT-PCR analysis revealed that ELF-EMF exposure to ES-derived neural cells significantly affected transcript levels of the apoptosis-related bcl-2, bax, and cell cycle regulatory "growth arrest DNA damage inducible" GADD45 genes, whereas mRNA levels of neural-specific genes were not affected. RF-EMF exposure of neural progenitor cells resulted in down-regulation of neural-specific Nurr1 and in up-regulation of bax and GADD45 mRNA levels. Short-term RF-EMF exposure

for 6 h, but not for 48 h, resulted in a low and transient increase of DNA double-strand breaks. No effects of ELF- and RF-EMF on mitochondrial function, nuclear apoptosis, cell proliferation, and chromosomal alterations were observed. We may conclude that EMF exposure of ES-derived neural progenitor cells transiently affects the transcript level of genes related to apoptosis and cell cycle control. However, these responses are not associated with detectable changes of cell physiology, suggesting compensatory mechanisms at the translational and posttranslational level.

Reese JA, Jostes RF, Frazier ME. Exposure of mammalian cells to 60-Hz magnetic or electric fields: analysis for DNA single-strand breaks. *Bioelectromagnetics*. 9(3):237-247, 1998. (NE)

Chinese hamster ovary (CHO) cells were exposed for 1 h to 60-Hz magnetic fields (0.1 or 2 mT), electric fields (1 or 38 V/m), or to combined magnetic and electric fields (2 mT and 38 V/m, respectively). Following exposure, the cells were lysed, and the DNA was analyzed for the presence of single-strand breaks (SSB), using the alkaline elution technique. No significant differences in numbers of DNA SSB were detected between exposed and sham-exposed cells. A positive control exposed to X-irradiation sustained SSB with a dose-related frequency. Cells exposed to nitrogen mustard (a known cross-linking agent) and X-irradiation demonstrated that the assay could detect cross-linked DNA under our conditions of electric and magnetic field exposures.

Robison JG, Pendleton AR, Monson KO, Murray BK, O'Neill KL. Decreased DNA repair rates and protection from heat induced apoptosis mediated by electromagnetic field exposure. *Bioelectromagnetics*. 23(2):106-112, 2002. (E)

In this study, we demonstrate that electromagnetic field (EMF) exposure results in protection from heat induced apoptosis in human cancer cell lines in a time dependent manner. Apoptosis protection was determined by growing HL-60, HL-60R, and Raji cell lines in a 0.15 mT 60 Hz sinusoidal EMF for time periods between 4 and 24 h. After induction of apoptosis, cells were analyzed by the neutral comet assay to determine the percentage of apoptotic cells. To discover the duration of this protection, cells were grown in the EMF for 24 h and then removed for 24 to 48 h before heat shock and neutral comet assays were performed. Our results demonstrate that EMF exposure offers significant protection from apoptosis ($P < .0001$ for HL-60 and HL-60R, $P < .005$ for Raji) after 12 h of exposure and that protection can last up to 48 h after removal from the EMF. In this study we further demonstrate the effect of the EMF on DNA repair rates. DNA repair data were gathered by exposing the same cell lines to the EMF for 24 h before damaging the exposed cells and non-exposed cells with H₂O₂. Cells were allowed to repair for time periods between 0 and 15 min before analysis using the alkaline comet assay. Results showed that EMF exposure significantly decreased DNA repair rates in HL-60 and HL-60R cell lines ($P < .001$ and $P < .01$ respectively), but not in the Raji cell line. Importantly, our apoptosis results show that a minimal time exposure to an EMF is needed before observed effects. This may explain previous studies showing no change in apoptosis susceptibility and repair rates when treatments and EMF exposure were

administered concurrently. More research is necessary, however, before data from this in vitro study can be applied to in vivo systems.

Scarfi MR, Sannino A, Perrotta A, Sarti M, Mesirca P, Bersani F. Evaluation of genotoxic effects in human fibroblasts after intermittent exposure to 50 Hz electromagnetic fields: a confirmatory study. Radiat Res. 164(3):270-276, 2005. (NE)

The aim of this investigation was to confirm the main results reported in recent studies on the induction of genotoxic effects in human fibroblasts exposed to 50 Hz intermittent (5 min field on/10 min field off) sinusoidal electromagnetic fields. For this purpose, the induction of DNA single-strand breaks was evaluated by applying the alkaline single-cell gel electrophoresis (SCGE)/comet assay. To extend the study and validate the results, in the same experimental conditions, the potential genotoxicity was also tested by exposing the cells to a 50 Hz powerline signal (50 Hz frequency plus its harmonics). The cytokinesis-block micronucleus assay was applied after 24 h intermittent exposure to both sinusoidal and powerline signals to obtain information on cell cycle kinetics. The experiments were carried out on human diploid fibroblasts (ES-1). For each experimental run, exposed and sham-exposed samples were set up; positive controls were also provided by treating cells with hydrogen peroxide or mitomycin C for the comet or micronucleus assay, respectively. No statistically significant difference was detected in exposed compared to sham-exposed samples in any of the experimental conditions tested ($P > 0.05$). In contrast, the positive controls showed a statistically significant increase in DNA damage in all cases, as expected. Accordingly, our findings do not confirm the results reported previously for either comet induction or an increase in micronucleus frequency.

Schmitz C, Keller E, Freuding T, Silny J, Korr H. 50-Hz magnetic field exposure influences DNA repair and mitochondrial DNA synthesis of distinct cell types in brain and kidney of adult mice. Acta Neuropathol (Berl). 107(3):257-264, 2004. (E)

Despite several recent investigations, the impact of whole-body magnetic field exposure on cell-type-specific alterations due to DNA damage and DNA repair remains unclear. In this pilot study adult mice were exposed to 50-Hz magnetic field (mean value 1.5 mT) for 8 weeks or left unexposed. Five minutes after ending exposure, the mice received [(3)H]thymidine and were killed 2 h later. Autoradiographs were prepared from paraffin sections of brains and kidneys for measuring unscheduled DNA synthesis and mitochondrial DNA synthesis, or in situ nick translation with DNA polymerase-I and [(3)H]dTTP. A significant ($P < 0.05$) increase in both unscheduled DNA synthesis and in situ nick translation was only found for epithelial cells of the choroid plexus. Thus, these two independent methods indicate that nuclear DNA damage is produced by long-lasting and strong magnetic field exposure. The fact that only plexus epithelial cells were affected might point to possible effects of magnetic fields on iron transport across the blood-cerebrospinal fluid barrier, but the mechanisms are currently not understood. Mitochondrial DNA synthesis was exclusively increased in renal epithelial cells of distal convoluted tubules and collecting ducts, i.e., cells with a very high content of mitochondria, possibly indicating increased metabolic activity of these cells.

Singh N, Lai H. 60 Hz magnetic field exposure induces DNA crosslinks in rat brain cells. *Mutat Res.* 400(1-2):313-320, 1998. (E)

In previous research, we found an increase in DNA strand breaks in brain cells of rats acutely exposed to a 60 Hz magnetic field (for 2 h at an intensity of 0.5 mT). DNA strand breaks were measured with a microgel electrophoresis assay using the length of DNA migration as an index. In the present experiment, we found that most of the magnetic field-induced increase in DNA migration was observed only after proteinase-K treatment, suggesting that the field caused DNA-protein crosslinks. In addition, when brain cells from control rats were exposed to X-rays, an increase in DNA migration was observed, the extent of which was independent of proteinase-K treatment. However, the X-ray-induced increase in DNA migration was retarded in cells from animals exposed to magnetic fields even after proteinase-K treatment, suggesting that DNA-DNA crosslinks were also induced by the magnetic field. The effects of magnetic fields were also compared with those of a known DNA crosslink-inducing agent mitomycin C. The pattern of effects is similar between the two agents. These data suggest that both DNA-protein and DNA-DNA crosslinks are formed in brain cells of rats after acute exposure to a 60 Hz magnetic field.

Stronati L, Testa A, Villani P, Marino C, Lovisolo GA, Conti D, Russo F, Fresegna AM, Cordelli E Absence of genotoxicity in human blood cells exposed to 50 Hz magnetic fields as assessed by comet assay, chromosome aberration, micronucleus, and sister chromatid exchange analyses. *Bioelectromagnetics.* 25(1):41-48, 2004. (NE)

In the past, epidemiological studies indicated a possible correlation between the exposure to ELF fields and cancer. Public concern over possible hazards associated with exposure to extremely low frequency magnetic fields (ELFMFs) stimulated an increased scientific research effort. More recent research and laboratory studies, however, have not been able to definitively confirm the correlation suggested by epidemiological studies. The aim of this study was to evaluate the effects of 50 Hz magnetic fields in human blood cells exposed *in vitro*, using several methodological approaches for the detection of genotoxicity. Whole blood samples obtained from five donors were exposed for 2 h to 50 Hz, 1 mT uniform magnetic field generated by a Helmholtz coil system. Comet assay, sister chromatid exchanges (SCE), chromosome aberrations (CA), and micronucleus (MN) tests were used to assess DNA damage, one hallmark of malignant cell transformation. The effects of a combined exposure with X-rays were also evaluated. Results obtained do not show any significant difference between ELFMFs exposed and unexposed samples. Moreover, no synergistic effect with ionizing radiation has been observed. A slight but significant decrease of cell proliferation was evident in ELFMFs treated samples and samples subjected to the combined exposure.

Svedenstal BM, Johanson KJ, Mild KH. DNA damage induced in brain cells of CBA mice exposed to magnetic fields. *In Vivo.* 13(6):551-552, 1999. (E)

DNA migration, using single cell gel electrophoresis (comet assay), was studied on brain

cells of CBA mice exposed continuously to 50 Hz, 0.5 mT magnetic fields (MF) for 2 hrs, 5 days or 14 days. No differences were observed in the groups MF-exposed for 2 hrs and 5 days compared with controls. However, in the group exposed to MF for 14 days, a significantly extended cell DNA migration was observed ($0.02 < p < 0.05$). These changes together with results from previous studies indicate that magnetic fields may have genotoxic effects in brain cells.

Testa A, Cordelli E, Stronati L, Marino C, Lovisolo GA, Freseigna AM, Conti D, Villani P. Evaluation of genotoxic effect of low level 50 Hz magnetic fields on human blood cells using different cytogenetic assays. Bioelectromagnetics. 25(8):613-619, 2004. (NE)

The question whether extremely low frequency magnetic fields (ELFMFs) may contribute to mutagenesis or carcinogenesis is of current interest. In order to evaluate the possible genotoxic effects of ELFMFs, human blood cells from four donors were exposed in vitro for 48 h to 50 Hz, 1 mT uniform magnetic field generated by a Helmholtz coil system. Comet assay (SCGE), sister chromatid exchanges (SCE), chromosome aberrations (CAs), and micronucleus (MN) test were used to assess the DNA damage. ELF pretreated cells were also irradiated with 1 Gy of X-ray to investigate the possible combined effect of ELFMFs and ionizing radiation. Furthermore, nuclear division index (NDI) and proliferation index (PRI) were evaluated. Results do not evidence any DNA damage induced by ELFMF exposure or any effect on cell proliferation. Data obtained from the combined exposure to ELFMFs and ionizing radiation do not suggest any synergistic or antagonistic effect.

Villarini M, Moretti M, Scassellati-Sforzolini G, Boccioli B, Pasquini R. Effects of co-exposure to extremely low frequency (50 Hz) magnetic fields and xenobiotics determined in vitro by the alkaline comet assay. Sci Total Environ. 361(1-3):208-219, 2006. (E)

In the present study, we used human peripheral blood leukocytes from 4 different donors, to investigate in vitro the possible genotoxic and/or co-genotoxic activity of extremely low frequency magnetic fields (ELF-MF) at 3 mT intensity. Two model mutagens were used to study the possible interaction between ELF-MF and xenobiotics: N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and 4-nitroquinoline N-oxide (4NQO). Primary DNA damage was evaluated by the alkaline single-cell microgel-electrophoresis ("comet") assay. Control cells (leukocytes not exposed to ELF-MF, nor treated with genotoxins) from the different blood donors showed a comparable level of basal DNA damage, whereas the contribution of individual susceptibility toward ELF-MF and the tested genotoxic compounds led to differences in the extent of DNA damage observed following exposure to the genotoxins, both in the presence and in the absence of an applied ELF-MF. A 3 mT ELF-MF alone was unable to cause direct primary DNA damage. In leukocytes exposed to ELF-MF and genotoxins, the extent of MNNG-induced DNA damage increased with exposure duration compared to sham-exposed cells. The opposite was observed in cells treated with 4NQO. In this case the extent of 4NQO-induced DNA damage was somewhat reduced in leukocytes exposed to ELF-MF compared to sham-exposed cells. Moreover, in cells exposed to ELF-MF an increased

concentration of GSH was always observed, compared to sham-exposed cells. Since following GSH conjugation the genotoxic pattern of MNNG and 4NQO is quite different, an influence of ELF-MF on the activity of the enzyme involved in the synthesis of GSH leading to different activation/deactivation of the model mutagens used was hypothesized to explain the different trends observed in MNNG and 4NQO genotoxic activity in the presence of an applied ELF-MF. The possibility that ELF-MF might interfere with the genotoxic activity of xenobiotics has important implications, since human populations are likely to be exposed to a variety of genotoxic agents concomitantly with exposure to this type of physical agent.

Williams PA, Ingebretsen RJ, Dawson RJ. 14.6 mT ELF magnetic field exposure yields no DNA breaks in model system *Salmonella*, but provides evidence of heat stress protection. *Bioelectromagnetics*. 27(6):445-450, 2006. (NE)

In this study, we demonstrate that common extremely low frequency magnetic field (MF) exposure does not cause DNA breaks in this *Salmonella* test system. The data does, however, provide evidence that MF exposure induces protection from heat stress. Bacterial cultures were exposed to MF (14.6 mT 60 Hz field, cycled 5 min on, 10 min off for 4 h) and a temperature-matched control. Double- and single-stranded DNA breaks were assayed using a recombination event counter. After MF or control exposure they were grown on indicator plates from which recombination events can be quantified and the frequency of DNA strand breaks deduced. The effect of MF was also monitored using a recombination-deficient mutant (*recA*). The results showed no significant increase in recombination events and strand breaks due to MF. Evidence of heat stress protection was determined using a cell viability assay that compared the survival rates of MF exposed and control cells after the administration of a 10 min 53 degrees C heat stress. The control cells exhibited nine times more cell mortality than the MF exposed cells. This *Salmonella* system provides many mutants and genetic tools for further investigation of this phenomenon.

Winker R, Ivancsits S, Pilger A, Adlkofer F, Rudiger HW. Chromosomal damage in human diploid fibroblasts by intermittent exposure to extremely low-frequency electromagnetic fields. *Mutat Res*. 585(1-2):43-49, 2005. (E)

Environmental exposure to extremely low-frequency electromagnetic fields (ELF-EMFs) has been implicated in the development of cancer in humans. An important basis for assessing a potential cancer risk due to ELF-EMF exposure is knowledge of biological effects on human cells at the chromosomal level. Therefore, we investigated in the present study the effect of intermittent ELF electromagnetic fields (50 Hz, sinusoidal, 5'field-on/10'field-off, 2-24 h, 1 mT) on the induction of micronuclei (MN) and chromosomal aberrations in cultured human fibroblasts. ELF-EMF radiation resulted in a time-dependent increase of micronuclei, which became significant after 10 h of intermittent exposure at a flux density of 1 mT. After approximately 15 h a constant level of micronuclei of about three times the basal level was reached. In addition, chromosomal aberrations were increased up to 10-fold above basal levels. Our data strongly indicate a clastogenic potential of intermittent low-frequency electromagnetic fields, which may lead to considerable chromosomal damage in dividing cells.

Wolf FI, Torsello A, Tedesco B, Fasanella S, Boninsegna A, D'Ascenzo M, Grassi C, Azzena GB, Cittadini A. 50-Hz extremely low frequency electromagnetic fields enhance cell proliferation and DNA damage: possible involvement of a redox mechanism. *Biochim Biophys Acta*. 1743(1-2):120-129, 2005. (E)

HL-60 leukemia cells, Rat-1 fibroblasts and WI-38 diploid fibroblasts were exposed for 24-72 h to 0.5-1.0-mT 50-Hz extremely low frequency electromagnetic field (ELF-EMF). This treatment induced a dose-dependent increase in the proliferation rate of all cell types, namely about 30% increase of cell proliferation after 72-h exposure to 1.0 mT. This was accompanied by increased percentage of cells in the S-phase after 12- and 48-h exposure. The ability of ELF-EMF to induce DNA damage was also investigated by measuring DNA strand breaks. A dose-dependent increase in DNA damage was observed in all cell lines, with two peaks occurring at 24 and 72 h. A similar pattern of DNA damage was observed by measuring formation of 8-OHdG adducts. The effects of ELF-EMF on cell proliferation and DNA damage were prevented by pretreatment of cells with an antioxidant like alpha-tocopherol, suggesting that redox reactions were involved. Accordingly, Rat-1 fibroblasts that had been exposed to ELF-EMF for 3 or 24 h exhibited a significant increase in dichlorofluorescein-detectable reactive oxygen species, which was blunted by alpha-tocopherol pretreatment. Cells exposed to ELF-EMF and examined as early as 6 h after treatment initiation also exhibited modifications of NF kappa B-related proteins (p65-p50 and I kappa B alpha), which were suggestive of increased formation of p65-p50 or p65-p65 active forms, a process usually attributed to redox reactions. These results suggest that ELF-EMF influence proliferation and DNA damage in both normal and tumor cells through the action of free radical species. This information may be of value for appraising the pathophysiologic consequences of an exposure to ELF-EMF.

Yaguchi H, Yoshida M, Ejima Y, Miyakoshi J. Effect of high-density extremely low frequency magnetic field on sister chromatid exchanges in mouse m5S cells. *Mutat Res*. 440(2):189-194, 1999. (E)

The induction of sister chromatid exchanges (SCEs) was evaluated in the cultured mouse m5S cells after exposure to extremely low frequency magnetic field (ELFMF; 5, 50 and 400 mT). Exposure to 5 mT and 50 mT ELFMF led to a very small increase in the frequency of SCEs, but no significant difference was observed between exposed and unexposed control cells. The cells exposed to 400 mT ELFMF exhibited a significant elevation of the SCE frequencies. There was no significant difference between data from treatments with mitomycin-C (MMC) alone and from combined treatments of MMC plus ELFMF (400 mT) at any MMC concentrations from 4 to 40 nM. These results suggest that exposure to highest-density ELFMF of 400 mT may induce DNA damage, resulting in an elevation of the SCE frequencies. We suppose that there may be a threshold for the elevation of the SCE frequencies, that is at least over the magnetic density of 50 mT.

Yokus B, Cakir DU, Akdag MZ, Sert C, Mete N. Oxidative DNA damage in rats exposed to extremely low frequency electro magnetic fields. *Free Radic Res*. 39(3):317-323, 2005. (E)

Extremely low frequency (ELF) electromagnetic field (EMF) is thought to prolong the life of free radicals and can act as a promoter or co-promoter of cancer. 8-hydroxy-2'-deoxyguanosine (8OHdG) is one of the predominant forms of radical-induced lesions to DNA and is a potential tool to assess the cancer risk. We examined the effects of extremely low frequency electromagnetic field (ELF-EMF) (50 Hz, 0.97 mT) on 8OHdG levels in DNA and thiobarbituric acid reactive substances (TBARS) in plasma. To examine the possible time-dependent changes resulting from magnetic field, 8OHdG and TBARS were quantitated at 50 and 100 days. Our results showed that the exposure to ELF-EMF induced oxidative DNA damage and lipid peroxidation (LPO). The 8OHdG levels of exposed group (4.39 ± 0.88 and 5.29 ± 1.16 8OHdG/dG.10⁵), respectively) were significantly higher than sham group at 50 and 100 days (3.02 ± 0.63 and 3.46 ± 0.38 8OHdG/dG.10⁵) ($p < 0.001$, $p < 0.001$). The higher TBARS levels were also detected in the exposure group both on 50 and 100 days ($p < 0.001$, $p < 0.001$). In addition, the extent of DNA damage and LPO would depend on the exposure time ($p < 0.05$ and $p < 0.05$). Our data may have important implications for the long-term exposure to ELF-EMF which may cause oxidative DNA damage.

Zmyslony M, Palus J, Jajte J, Dziubaltowska E, Rajkowska E. DNA damage in rat lymphocytes treated in vitro with iron cations and exposed to 7 mT magnetic fields (static or 50 Hz). *Mutat Res.* 453(1):89-96, 2000. (E)

The present study was undertaken to verify a hypothesis that exposure of the cells to static or 50 Hz magnetic fields (MF) and simultaneous treatment with a known oxidant, ferrous chloride, may affect the oxidative deterioration of DNA molecules. The comet assay was chosen for the assessment of DNA damage. The experiments were performed on isolated rat lymphocytes incubated for 3h in Helmholtz coils at 7 mT static or 50 Hz MF. During MF exposure, part of the cell samples were incubated with 0.01 microM H_2O_2 and another one with 10 microg/ml $FeCl_2$, the rest serving as controls. Lymphocyte exposure to MF at 7 mT did not increase the number of cells with DNA damage in the comet assay. Incubation of lymphocytes with 10 microg/ml $FeCl_2$ did not produce a detectable damage of DNA either. However, when the $FeCl_2$ -incubated lymphocytes were simultaneously exposed to 7 mT MF, the number of damaged cells was significantly increased and reached about 20% for static MF and 15% for power frequency MF. In the control samples about 97% of the cells did not have any DNA damage. It is not possible at present to offer a reasonable explanation for the findings of this investigation - the high increase in the number of lymphocytes showing symptoms of DNA damage in the comet assay, following simultaneous exposure to the combination of two non-cytotoxic factors - 10 microg/ml $FeCl_2$ and 7 mT MF. In view of the obtained results we can only hypothesise that under the influence of simultaneous exposure to $FeCl_2$ and static or 50 Hz MF, the number of reactive oxygen species generated by iron cations may increase substantially. Further studies will be necessary to confirm this hypothesis and define the biological significance of the observed effect.

Zmyslony M, Palus J, Dziubaltowska E, Politanski P, Mamrot P, Rajkowska E, Kamedula M. Effects of in vitro exposure to power frequency magnetic fields on UV-induced DNA damage of rat lymphocytes. Bioelectromagnetics. 25(7):560-562, 2004. (E)

The mechanisms of biological effects of 50/60 Hz (power frequency) magnetic fields (MF) are still poorly understood. There are a number of studies indicating that MF affect biochemical processes in which free radicals are involved, such as the biological objects' response to ultraviolet radiation (UVA). Therefore, the present study was aimed to assess the effect of 50 Hz MFs on the oxidative deterioration of DNA in rat lymphocytes irradiated in vitro by UVA. UVA radiation (150 J/m²) was applied for 5 min for all groups and 50 Hz MF (40 microT rms) exposure was applied for some of the groups for 5 or 60 min. The level of DNA damage was assessed using the alkaline comet assay, the fluorescence microscope, and image analysis. It has been found that the 1 h exposure to MF caused an evident increase in all parameters consistent with damaged DNA. This suggest that MF affects the radical pairs generated during the oxidative or enzymatic processes of DNA repair.

SECTION 7

Evidence for Stress Response (Stress Proteins)

Health Risk of Electromagnetic Fields: Research on the Stress Response

**Martin Blank, PhD
Department of Physiology and Cellular Biophysics
College of Physicians and Surgeons
Columbia University**

**Prepared for the BioInitiative Working Group
July 2007**

A Scientific Perspective on Health Risk of Electromagnetic Fields: Research on the Stress Response

Table of Contents

	Page
I. Abstract	2
II. Stress Proteins – Conclusions	2
III. ELF and RF activation of the stress response	4
IV. DNA activation mechanisms: EMFs and electrons	7
V. The critical role of scientific research	10
VI. The troubling context of today's science	
VII. Replication and failures to replicate experimental results	15
VIII. A critical look at a recent review of the stress response	18
IX. Rethinking EMF safety in a biology context	21
X. Summary	22
XI. References	23
XII. Tables	
Table 1 Studies of EMF Stimulation of DNA and Protein Synthesis	33
Table 2 Biological Thresholds in the ELF Range	38

I. Abstract

The stress response is a protective cellular mechanism that is characterized by stress protein synthesis. The stress response, by its very nature, shows that *cells react to EMFs as potentially harmful*. The stress response is an important protective mechanism that enables cells from animals, plants and bacteria to survive environmental stressors with the aid of heat shock proteins (hsp). It is stimulated by both non-thermal power (ELF), and non-thermal radiofrequency (RF) as well as thermal radio (RF) frequency EMFs, so the greatly differing energies are not critical in activating the DNA to synthesize proteins. Direct interaction of both ELF and RF EMFs with DNA is likely, since specific DNA sequences are sensitive to EMFs and retain their sensitivity when transferred to artificial molecular constructs. Basic science research is essential for determining the biological parameters needed to assess health risks of electromagnetic fields (EMFs) and the molecular mechanisms that explain them. However, the adversarial nature of the debate about risk has clouded the evaluation of the science. To clarify the results of research on EMF stimulation of the stress response, it is necessary to consider the scientific context as well as the research. There is ample evidence that ELF and RF fields activate DNA in cells and cause damage at exposure levels that are considered 'safe' (i.e., below current exposure limits that are based on tissue heating as measured in Specific Absorption Rate or SAR). Because non-thermal EMFs are biologically active and potentially harmful, new safety standards must be developed to protect against possible damage at non-thermal levels, and the standards must be defined in terms of a non-thermal biological dose. Fewer than one quarter of the relevant references listed in Table 1 appear in the IEEE list leading to the newly revised IEEE C95.1 recommendations (April, 2006).

II. Stress Proteins - Conclusions (Heat Shock Proteins)

Conclusion: *Scientific research has shown that the public is not being protected from potential damage that can be caused by exposure to EMF, both power frequency (ELF) and radio frequency (RF).*

Conclusion: *DNA damage (e.g., strand breaks), a cause of cancer, occurs at levels*

of ELF and RF that are below the safety limits. Also, there is no protection against cumulative effects stimulated by different parts of the EM spectrum.

Conclusion: *The scientific basis for EMF safety limits is flawed when the same biological mechanisms are activated in ELF and RF ranges at vastly different levels of the Specific Absorption Rate (SAR). Activation of DNA to synthesize stress proteins (the stress response), is stimulated in the ELF at a non-thermal SAR level that is over a billion times lower than the same process activated in the RF at the thermal level.*

Conclusion: *There is a need for a biological standard to replace the thermal standard and to also protect against cumulative effects across the EM spectrum.*

III. ELF and RF activation of the stress response

Much detailed information about the stress response will be presented in the following sections and in the tables, but the most important finding to keep in mind is that *both ELF and RF fields activate the synthesis of stress proteins*. All cells do not respond to EMF, but activation of the same cellular mechanism by both thermal and non-thermal stimuli in a variety of cells shows that both ELF and RF are biologically active and that a biological ‘dose’ of EMF cannot be described in terms of SAR (Blank and Goodman, 2004a). SAR is irrelevant for non-thermal ELF responses, where energy thresholds are many orders of magnitude lower than in RF. A new definition of EMF dose is necessary for describing a safety limit, and SAR must be replaced by a measure of exposure that can be defined in biological terms.

The stress response, by its very nature, shows that *cells react to EMFs as potentially harmful*. The stress response is an important protective mechanism that enables cells from animals, plants and bacteria to survive environmental stressors, such as sharp increases in temperature (originally called ‘heat shock’), hypoxia, and dissolved toxic heavy metals like Cd^{+2} and oxidative species that can damage proteins and DNA (‘oxidative stress’). The stress response is evolutionarily conserved in essentially all eukaryotic and prokaryotic organisms, but not all stressors are effective in all cells, and different stress proteins are activated under different conditions. Stress proteins are a family of about 20 different proteins, ranging in size from a few kilodaltons to over 100kD. The 27kD and 70kD protein families are the most common and most frequently studied.

Kültz (2005) has called the stress response a ‘... defense reaction of cells to damage that environmental forces inflict on macromolecules.’, based on evidence from gene analysis showing that the stress response is a reaction to molecular damage. The genes activated as a group along with stress genes, which Kültz calls the ‘universally conserved proteome’, are those associated with sensing and repairing damage to DNA and proteins.

Stress proteins help damaged proteins refold to regain their conformations, and also act as “chaperones” for transporting cellular proteins to their destinations in cells. The molecular damage stimulated by non-thermal ELF fields occurs in the absence of an increase in temperature. ELF energy thresholds are estimated to be about 10^{-12} W/kg, over a billion times lower than the thermal stimuli that cause damage in the RF range (Blank and Goodman, 2004a).

The classic stress response to a sharp increase in temperature (i.e., ‘heat shock’) is associated with a biochemical pathway where transcription factors known as heat shock factors, HSFs, translocate from the cytoplasm to the nucleus, trimerize and bind to DNA at the heat shock elements (HSEs) in the promoters of the genes. The promoter is the DNA segment where protein synthesis is initiated and it is not part of the coding region. The HSEs contain specific nucleotide sequences, nGAAn, that are the consensus sequences for thermal stimuli. The binding of HSFs to HSEs, etc is similar for heat shock in plant, animal and bacterial cells. ELF range EMFs have been shown to follow the same sequence of events in inducing stress response proteins in human cells, including breast (MCF7, HTB124), leukemia (HL60), epithelial cells, as well as *E. coli* and yeast cells.

Studies done with chick embryos and cells from *Drosophila* and *Sciara* salivary gland chromosomes have produced graphic evidence of the effects of EMF. In *Drosophila* and *Sciara* salivary gland chromosomes, EMF causes the formation of ‘puff’s, enlarged regions along the chromosome, at loci associated with activation of heat shock genes. This is followed by elevated concentrations of transcripts at the sites and eventually stress protein synthesis (Goodman and Blank, 1998). The changes in chromosome morphology are characteristic of the stress response to both EMF and elevated temperature. Chick embryos develop hearts that stop beating when the oxygen concentration is lowered, but that can be protected and kept beating if stress proteins have been induced by ELF fields (DiCarlo et al, 1998) and in the RF range (Shallom et al, 2002).

The cellular response pathways to EMF have been characterized in the ELF range (Goodman and Blank, 2002), and have been found to share some of the characteristics of heat shock stress, such as the movement of heat shock factor monomers from the cytoplasm to the nucleus. The biochemical mechanism that is activated, the MAPK signaling pathway, differs from the thermal pathway (Goodman and Blank, 2002), but is the same as the non-thermal pathway in the RF range (Leszczynski et al, 2002).

The HSP70 gene is activated within minutes in cells exposed to ELF fields (Lin et al, 1997), and is accompanied by the binding of HSFs to the specific nucleotide sites in the promoter of the gene. However, different segments of the DNA promoter function as HSEs. Research in the ELF range has shown that the promoter of the major stress protein, hsp70, has two domains that respond to two different physical stimuli, EMF and an increase in temperature (Lin et al, 1999). The stimulus-specific domains have different DNA sequences that cannot be interchanged. The ***DNA consensus sequences that respond to EMF are nCTCTn*** (Lin et al, 1997; 1999). These differ from the nGAAn consensus sequences for thermal stimuli. The existence of two different consensus sequences that respond to EMF and temperature increase, respectively, are molecular

evidence of different pathways that respond to non-thermal and thermal stimuli.

In another series of experiments, a DNA sequence from the promoter of an EMF sensitive gene was included in a construct containing a reporter gene, either chloramphenicol amino transferase (CAT) or luciferase. In each case, the construct proved to be EMF sensitive and reacted when an ELF field was applied (Lin et al, 2001). The ability to transfer EMF sensitive DNA sequences that subsequently respond to an EMF is further evidence linking the cellular response to a DNA structure.

In heat shock, the stress response is activated when extracellular signals affect receptors in the plasma membrane. This probably does not happen with an EMF, which can easily penetrate throughout the cell and whose actions are therefore not limited to the membrane. One can transfer the EMF response by transferring the DNA consensus sequences (Lin et al, 2001), so it is likely that the activation mechanism involves direct EMF interaction with the DNA consensus sequences. The cell based signal transduction pathways of the heat shock response are involved in regulation of the EMF stimulated process, probably through the feedback control mechanisms that respond to the stress proteins synthesized or the mRNA concentrations that code for them (Lin et al, 1998).

Repeated induction of the stress response in a cell has been shown to induce cytoprotection, a reduced response associated with restimulation (Blank and Goodman, 1998). This is analogous to thermotolerance, the reduced response to an increase in temperature after an initial heat shock response. Experiments with developing chick embryos show similar habituation to repeated stimulation in the ELF range (DiCarlo et al, 2002). There are different effects of continuous and intermittent EMF exposures that show feedback control features in the EMF stimulated stress response (Lin et al, 1997). This autoregulatory reaction is an indication that the thermotolerance mechanism is inherent in the response to a single stimulus as well.

It has now been shown in many laboratories that RF also stimulates the cellular stress response and cells start to synthesize stress proteins in many different kinds of cells (e.g., Kwee et al, 2001; Shallom et al, 2002; Leszczynski et al, 2002; Weisbrot et al, 2004). Cotgreave (2005) included many cells that did not synthesize stress proteins in response to RF stimulation in his summary of data. The listings in Table 1 contain additional positive and negative results. It is quite clear that certain cell lines do not respond to EMF by synthesizing stress proteins. The reasons are not known, but the changes in cells in tissue culture and in cancer cells may render some of them unable to respond to EMF. In addition to mutations in cell lines, pre-exposure to ambient ELF and RF fields in the laboratory can also affect an ability to respond. What we can say in summary at this stage is that:

- the stress response has been demonstrated in many cells and linked to changes in the DNA and chromosomes.

- there are similarities in stress protein synthesis stimulated in the non-thermal ELF and thermal RF frequency ranges.
- the biochemical mechanism that is activated is the same non-thermal pathway in both ELF and RF, and is not associated with the thermal response.

IV. DNA activation mechanisms: EMFs and electrons

We think of DNA as a very stable polymer that stores and transmits genetic information from generation to generation. However, DNA must also come apart relatively easily to enable the continuous protein synthesis that is needed to sustain living cells. Usually, this process is started when specialized proteins called transcription factors bind to DNA. However, both ELF and RF fields also stimulate DNA to start protein synthesis. EMF stimulation of stress protein synthesis indicates activation of DNA, even by relatively weak non-thermal ELF. This raises the possibility that EMF can cause other changes in DNA that interfere with the copying and repair processes in DNA, and that can lead to mutations and cancer.

Protein synthesis starts when the two chains of DNA come apart to make an mRNA copy of the amino acid code for a particular protein. This occurs at the specific DNA segment where the transcription factor binds, and in forming a bond changes the electron distribution. Since recent research has shown electron conduction in DNA (Wan et al, 1999; 2000; Ratner, 1999; Porath et al, 2000; Giese and Spichty, 2000), it is possible that EMF affects electron distribution and movement in DNA, and helps it to come apart to initiate protein synthesis, not unlike the action of a transcription factor. Charge transport through DNA depends on the DNA sequence (Shao et al, 2005), and there are reasons to believe that EMFs would cause the DNA to come apart at the EMF consensus sequence, nCTCTn (Blank and Goodman, 2002).

The ability of relatively small perturbations to stimulate DNA to initiate biosynthesis is consistent with larger perturbations that lead to DNA strand breaks. Several experimental studies have reported both single and double strand breaks in DNA and other chromosome damage after exposure to ELF fields (Lai and Singh, 1997a; Ivancsits et al, 2005; Diem et al, 2005; Winker et al, 2005). Ivancsits et al (2005) found DNA damage in fibroblasts, melanocytes and rat granulosa cells, but not in lymphocytes, monocytes and skeletal muscle cells. Single and double strand breaks and other DNA damage after exposure to RF fields have also been reported (Phillips et al, 1998; Sarimov et al, 2004; Lai and Singh, 2005).

The Ivancsits, Diem and Winker studies cited above are part of the REFLEX Project, a collaboration of twelve laboratories in seven countries of the European Union (REFLEX, 2004). The group found that both ELF and RF exposures, below the current safety limits, modified the expression of many genes and proteins. They also reported DNA damage (e.g., strand breaks, micronuclei, chromosomal damage) due to ELF fields at exposures

of 35 μ T. Similar genotoxic effects were produced in fibroblasts, granulosa cells and HL60 cells by RF fields at SARs between 0.3 and 2W/kg. The expression and phosphorylation of the stress protein hsp27 was one of the many proteins affected.

The REFLEX Project Report (2004) is available on the internet and well worth consulting as a source of much information about the effects on cells *in vitro* due to the ELF and RF exposures we encounter in our environment. The Report has an introduction by Ross Adey, one of the last things he wrote, telling us about the importance of establishing "...essential exposure metrics ... based on mechanisms of field interactions in tissues". One needs a biological metric in order to characterize EMF exposure.

The possibility that EMFs could cause greater damage to DNA in the RF range and at longer exposures was demonstrated by Phillips et al (1998) who reported more DNA breaks when cells were exposed at higher SARs. They suggested that the rate at which DNA damage can be repaired (or eliminated by apoptosis) is limited, and when the rate of damage at the higher SARs exceeds the repair rate, there is the possibility of retaining mutations and initiating carcinogenesis. Chow and Tung (2000) reported that exposure to a 50Hz magnetic field enhances DNA repair through the induction of DnaK/J synthesis. The eternal struggle in cells and organisms between the forces tending to break things down (catabolism) and those tending to build up and repair (anabolism) probably accounts for much of the variability one finds in experiments with cells as well as with people.

The changes in DNA initiated by ELF fields cannot be explained by thermal effects. Electric and magnetic fields interact with charges and magnetic dipoles, and fundamental mechanisms must ultimately be based on these interactions. From the data in Table 2, it is clear that relatively little energy is needed for effects on electron transfer (Blank and Goodman, 2002; 2004b; Blank, 2005). The low energies needed to perturb DNA in the ELF range suggest that the mechanism involves electrons, e.g., probably in the H-bonds that hold the two chains of DNA together. Electrons have very high charge to mass ratio and are most likely to be affected even by weak electric and magnetic fields.

There are many indications that electrons are involved in EMF reactions with DNA. In experiments that stimulate the stress response, the estimated force of $\sim 10^{-21}$ newtons that activates DNA can move a free electron about the length of a H-bond (~ 0.3 nm) in 1ns. The calculated electron velocity is comparable to electron velocities measured in DNA (Wan et al, 1999; 2000), and is also expected if electrons move at the \sim nanometer/picosecond flickering rate of protons in H-bonded networks (Fecko et al, 2003) that would be present at normally hydrated DNA sites. Electrons can tunnel nanometer distances in proteins (Gray and Winkler, 2003), and experiments have shown comparable electron movement in DNA (Wan et al, 1999; 2000). Electrons might be expected to move more readily from the CTCT bases in the consensus sequence, because of their low electron affinities. Finally, ELF fields have been shown to accelerate electron transfer in oxidation-reduction reactions (Blank and Soo, 1998; 2003).

The fact that the same non-thermal mechanism is activated in ELF and RF ranges

emphasizes that it is not the total energy associated with the EMF that is critical, but rather the regular oscillations of the stimulating force. As already mentioned earlier, the energy associated with each wave (i.e., energy/cycle) is more or less independent of the frequency. If the same energy is needed to reach threshold in both ELF and RF, the many repetitions at the higher frequency cause the non-thermal threshold to be reached in a shorter time and the total energy absorbed over time to increase with frequency. Even in the ELF range, where SAR levels are very low, the stress response is activated by short exposures to fields of less than $1\mu\text{T}$, while single and double strand breaks in DNA have been reported at longer exposures to higher field strengths $\sim 0.1\text{mT}$ (Lai and Singh, 2005). The two mechanisms appear to be related in that breaks in DNA appear to result from free radical mechanisms that also involve electron transfer reactions (Lai and Singh, 1997b).

The reaction of EMFs with DNA differs from those listed in Table 2 in that they appear to occur with equal ease at the widely differing frequencies in ELF and RF ranges. The frequency dependence of a reaction provides information about how time constants of charge transfer processes are affected by fields, and the frequency responses of the few EMF sensitive biological systems that have been studied suggest that fields are most effective at frequencies that are close to the natural rhythms of the processes affected (Blank and Soo, 2001a; Blank and Goodman, 2004b; Blank, 2005). Frequency optima for the enzymes, Na,K-ATPase and cytochrome oxidase, differ by an order of magnitude with maximums at about 60Hz and 800Hz, respectively (Blank and Soo, 2001a), in both cases close to the observed frequency maximum of the enzyme reaction. The rate constant of the BZ reaction is about 250Hz, the frequency of the rate limiting step in a multi-step process with at least 10 sub-reactions (Blank and Soo, 2003).

The electrons in DNA that are affected by EMFs are probably not engaged in electron transfer reactions. They respond to frequencies that range from ELF to RF and are more likely to be tied to the wide frequency range of fluctuations than to the frequency of a particular reaction. The displacement of electrons in DNA would charge small groups of base pairs and lead to disaggregation forces overcoming H-bonds, separating the two chains and enabling transcription. Studies have shown that biopolymers can be made to disaggregate when the molecular charge is increased (Blank, 1994; Blank and Soo, 1987). This explanation would also apply to the effect of applied electric fields that also activate DNA. Electric fields exert a force on electrons, and have been shown to stimulate protein synthesis in HL60 cells (Blank et al, 1992), E coli (Laubitz et al, 2006) and muscle *in vivo* (Blank, 1995). The genes for the hsp70 stress protein are more likely to be activated since they have been shown to be 'bookmarked' on the DNA chain, that is, more exposed to externally applied forces (Xing et al, 2005).

The outline of a plausible mechanism to account for EMF activation of DNA through interaction with electrons has relied on evidence from many lines of research. This mechanism may or may not hold up under further testing, but the experimental facts it is based on have been verified. It has been clearly demonstrated that exposure of cells to non-thermal power and thermal radio frequency EMFs, at levels deemed to be safe for human exposure, activate DNA production of stress proteins and could increase the

number of DNA breaks. There is ample experimental evidence to support the possibility of DNA damage at non-thermal levels of exposure, and the need for greater protection.

V. The critical role of scientific research

The connection between the results of scientific research and assessing EMF risk does not appear to be working well. We all agree that EMFs are unsafe at the level where they cause electrocution, and that we must protect against that possibility. We also agree that if other risks are associated with EMFs, we must identify them and determine the exposure levels at which they occur. This task requires that we define a biological dose of EMF, and that we obtain information about cellular mechanisms activated at different doses. As we have seen, the currently accepted measure of EMF dose, the specific absorption rate (SAR), is definitely not a measure of the effective biological dose when stress protein synthesis can be stimulated by SAR levels that differ by many orders of magnitude in the ELF and RF ranges (Blank and Goodman, 2004a). Yet, there is strong opposition to accepting the consequences of these experimental facts.

Regarding EMF mechanisms, we still have much to learn, but we know that the energy and field strength thresholds of many biological reactions are very low (Table 2). These findings indicate that safe exposure levels for the public should be substantially lowered, if only as a precautionary measure. Even when stated in vague terms, so as to require little more than lip service, a precautionary policy has not yet been recommended by the WHO. Thus, the two main problems of research on EMF risk, defining a biological dose and the desired level of exposure protection, remain to be solved.

Scientific research can contribute to defining a biological dose, but the desired level of exposure protection is a more complicated issue. Guidance for EMF policy on exposure protection has come primarily from epidemiology studies of health risks associated with power lines in the case of ELF, and cell phones in the case of RF. Basic research studies do not provide insight into the effects of long term exposures that are so important in determining risk, and they appear to have been used almost entirely to probe biochemical mechanisms that might underlie health risks identified in epidemiology studies. However, the research does overcome a basic weakness of epidemiology studies, an inability to determine a causal relation and to rule out effects of possible confounders. Epidemiology studies can correlate EMF exposure and health effects in human populations, and show quantitative dose-response relations, but it is only when coupled with basic research on molecular mechanisms that one can test and establish the scientific plausibility of effects of exposure. This scientific capability has become more important with recent advances in research on DNA, where mutations associated with initiation and promotion of cancer can be identified. EMF laboratory research has also played an indirect role in the practical aspects of risk by showing that:

- many biological systems are affected by EMFs,
- EMFs compete with intrinsic forces in a system, so effects can be variable,

- many frequencies are active,
- field strength and exposure duration thresholds are very low,
- molecular mechanisms at very low energies are plausible links to disease (e.g., effect on electron transfer rates linked to oxidative damage, DNA activation linked to abnormal biosynthesis and mutation).

Research on the stress response, a protective mechanism that involves activation of DNA and protein synthesis, was not included in previous scientific reviews prior to evaluating safety standards, and thus provides additional insights into EMF interactions (Blank and Goodman, 2004a). Activation of this protective mechanism by non-thermal as well as thermal EMF frequencies has demonstrated:

- the reality and importance of non-thermal effects of EMFs,
- that cells react to an EMF as potentially harmful,
- the same biological reaction to an EMF can be activated in more than one division of the EM spectrum,
- direct interaction of ELF and RF with DNA has been documented and both activate the synthesis of stress proteins,
- the biochemical pathway that is activated is the same pathway in both ELF and RF and it is non-thermal,
- thresholds triggering stress on biological systems occur at environment levels on the order of 0.5 to 1.0 μT for ELF,
- many lines of research now point to changes in DNA electron transfer as a plausible mechanism of action as a result of non-thermal ELF and RF.

Given these findings, the *specific absorption rate (SAR)* is not the appropriate measure of biological threshold or dose, and should not be used as a basis for a safety standard since it regulates against thermal effects only.

Cellular processes are unusually sensitive to non-thermal ELF frequency fields. The thresholds for a number of biological systems are shown in Table 2, and many are in the range of 0.5 to 1.0 μT , not very much higher than the usual environmental backgrounds of $\sim 0.1 \mu\text{T}$. The low biological thresholds in the non-thermal ELF range undermine claims that an EMF must increase the temperature in order to cause changes in cells. They also show that many biochemical reactions can be affected by relatively low field strengths, similar to those in the environment. -Non-thermal ELF fields can also cause DNA damage, and therefore add to health and safety concerns.

In addition to very low thresholds, exposure durations do not have to be very long to be effective. Litovitz et al (1991, 1993), working with the enzyme ornithine decarboxylase,

have shown a full response to an EMF when cells were exposed for only 10sec. This occurred with ELF sine waves or ELF modulated 915MHz sine waves. The exposure had to be continuous, since gaps in the sine wave resulted in a reduced response. Interference with the sine wave in the form of superimposed ELF noise also reduced the response (Mullins et al, 1998). The interfering effect of noise has been shown in the RF range by Lai and Singh (2005), who reported that noise interferes with the ability of an RF signal to cause breaks in DNA strands. The decreased effect when noise is added to a signal is yet another indication that EMF energy is not the critical factor in causing a response.

The finding that the stress response threshold can be stimulated in both ELF and RF frequency ranges appears to suggest that the threshold is independent of EMF energy. Energy increases with the frequency, so compared to an ELF energy of ~ 1 a.u. (arbitrary unit of energy), the energy at RF is $\sim 10^{11}$ a.u. Actually, it is the energy/cycle that is independent of frequency. A typical ELF cycle at 10^2 Hz lasts 10^{-2} sec and a typical RF cycle at 10^{11} Hz lasts 10^{-11} sec. Because the energy is spread over a different number of cycles each second in the two ranges, the same value of $\sim 10^{-2}$ a.u./cycle applies to both ELF and RF ranges.

An early review of the stress response in the ELF range (Goodman and Blank, 1998) summarized basic findings, and a more recent review by Cotgreave (2005) has provided much additional information, primarily on the RF range. Table 1 summarizes both ELF and RF studies (mainly frequencies 50Hz, 60Hz, 900MHz, 1.8GHz) relevant to stimulation of DNA and stress protein synthesis in many different cells. The list is not exhaustive, but the citations represent the different frequencies and biological systems, as well as the diversity of results in the literature. As already noted by Cotgreave (2005), the stress response does not occur in reaction to EMFs in all cells. A paper by Jin et al (2000), to be discussed later, shows that even the same cell line can give opposite results in the same laboratory. The stress response is an important topic in its own right, but its importance for EMF research is that it offers insights into EMF interaction mechanisms in the stimulation of DNA. On the practical level, the stress response has shown the need to replace the SAR standard to take into account non-thermal biological effects.

Differences in experimental results shown in Table 1 are not uncommon when studying phenomena that are not as yet well understood, and this frequently gives rise to controversy. In EMF research, however, other factors have contributed to a controversial scientific atmosphere. The following sections on the scientific context, as well as a critique of the review by Cotgreave, will show how discussion of the stress response and the absence of discussion on related topics have compromised the evaluation of the science. The discussion of stress response stimulation in ELF and RF ranges together with ideas on DNA mechanisms, has important implications regarding EMF risk and safety.

VI. The troubling context of today's science

The need to include basic research findings in assessment of health risks is clear, but it is

equally important to make sure that these findings are properly evaluated. No less an authority on science than Donald Kennedy (2006), the current Editor of *Science*, wrote "...how competitive the scientific enterprise has become, and the consequential incentive to push (or shred) the ethical envelope". He was referring primarily to the controversial religious/ political atmosphere over such issues as evolution, stem cell research, etc, but he could just as easily have included economic factors. In the following quote, editors of the *Journal of the American Medical Association* (JAMA 284:2203-2208, 2000) pointed out distortions in the proof of effectiveness of drugs in studies supported by the drug industry:

"There is a growing body of literature showing that faculty who have industry ties are more likely to report results that are favorable to a corporate sponsor, are more likely to conduct research that is of lower quality, and are less likely to disseminate their results to the scientific community".

Even *The Wall Street Journal* (Jan 9, 2007), which generally presents favorable views of business, had a front page article on the controversy over whether mycotoxins produced by molds are harmful, that was critical of scientist-business community connections. They pointed out that some scientific experts in the professional societies, who had issued statements minimizing harmful effects, had not disclosed their links to companies defending lawsuits in this area.

The connection between scientific expertise, the research that is done, and the source of support, has always been an ethical gray area, but the above examples and recent instances of experimental fraud have reinforced the impression that the ethical standards of scientists have deteriorated considerably. In our area of interest, insufficient attention has been paid to the influence the power and communication industries may be having on the research of those assessing EMF safety. At the Third International Standard Setting Seminar (October 2003) in Guilin, China, Prof. Henry Lai of the University of Washington summarized 179 cell phone studies showing that independent researchers were twice as likely to report biological effects due to RF in comparison to those funded by industry. This was very much in line with the earlier JAMA comment on the drug industry. Published reports have started to appear (Hardell et al, 2006; Huss et al, 2007) documenting the correlation of EMF research outcome with the source of support. Recognition of the phenomenon is a first step toward minimizing abuses, and one hopes that this information will eventually be factored into evaluation of the experimental results. I am not overly optimistic, since those who wish their influence to remain hidden can channel support through unaffiliated committees with non-committal names.

Science is a cooperative enterprise in the long run, but in day-to-day practice, there has always been competition among scientists for recognition and support. In EMF research, the atmosphere has become especially adversarial in the selection of participants and subjects to be covered in recent evaluations. Two important examples are the International Committee on Electromagnetic Safety (ICES) and IEEE sponsored symposium on "Reviews of Effects of RF Energy on Human Health" (BEMS Supplement 6, 2003), and the more recent WHO sponsored symposium "Sensitivity of Children to

EMF Exposure” (BEMS Supplement 7, 2005). Both collections of papers appeared in *Bioelectromagnetics*, the journal of the primary research society in this scientific specialty, where publication carries a certain aura of authority in the field. Of course, one expects the highest of ethical standards, and the editor assured everyone that normal reviewing procedures, etc, had been followed. However, all that had come after the scope of the papers had been narrowly defined so that there was no coverage of recent research on the EMF stimulated stress response or stimulation of DNA to initiate protein synthesis. An older mind set pervaded the choice of the topics and the papers. That mind set appeared to be stuck in the belief that non-thermal EMF was biologically inert, that the nucleus was an impregnable structure that unlocked the genetic information in its DNA only at the time of cell division, etc. These two meetings took place only a few years ago, in a world of science where it had already been known for some time that biochemical signals are continuously changing DNA in cell nuclei and mitochondria, turning on protein synthesis, checking and repairing DNA itself, etc. Research on the stress response had even shown that DNA was unusually sensitive to EMF by finding responses in the non-thermal ELF range. One expects to find such papers in symposia organized by the Mobile Manufacturers Forum, but not in *Bioelectromagnetics*.

A science based evaluation process cannot limit its scope of interest so as to ignore a research area that is so central in biology today, and that is obviously affected by EMF. Information on the EMF stimulated stress response and stimulation of DNA to initiate protein synthesis must be an integral part of the evaluation process, and its omission in earlier evaluations compromised the scientific basis of those reviews and distorted their conclusions.

It is ironic that the review in *Bioelectromagnetics* Supplement 6 listed as its first guiding principle that “The RF safety standard should be based on science”, essentially a reaffirmation of the IEEE guideline for the revision of C95.1-1991 safety standards. Scientific research is designed to answer questions, and answers do not come from deciding *a priori* that certain types of studies are not relevant or can be ignored because they have not been adequately proven in the eyes of the organizers. Scientific method is not democratic. The word ‘proof’ in ‘scientific proof’ is best understood in terms of its older meaning of ‘test’. It does not rely on an adversarial ‘weight of the evidence’, where opposing results and arguments are presented and compared. Answers do not come from keeping a scoreboard of positive versus negative results and merely tallying the numbers to get a score. In scientific proof, number and weight do not count. It is hard to see how the review in *Bioelectromagnetics* Supplement 6 could reconcile its advocacy of science as a guiding principle with its subsequent endorsement of “the weight of evidence approach” to be used in their assessment.

We should be reminded that ‘scientific proof’ is not symmetric (Popper, 1959). One cannot prove that EMF is harmless no matter how many negative results one presents. One single reproducible (significant) harmful effect would outweigh all the negative results.

The above characteristics of science are generally acknowledged to be valid as abstract

principles, but in EMF research, it has been quite common to list positive and negative findings and thereby imply equal weights. Table 1 is an alphabetical listing by first author of positive and negative findings, with the negative studies indicated as **NO** in bold. There is no scoreboard, since the studies are on many different systems, etc, and not of the same quality. The listing is not meant to be complete or to be scored, but rather to present the variety of biological systems studied in the different EMF ranges. Negative studies play an important role in science, and there is good reason to publish them when they are failures to replicate earlier positive results. This can often lead to important clarifications of the effect, the technique, etc. However, negative studies are being used in another way. Although they cannot prove there is no positive effect, they do have an influence in the unscientific 'weight of evidence approach'. In epidemiology, where it is difficult to compare studies done under different conditions, it is common to make a table of the positive and negative results. The simple listing has the effect of a tally, and the overall score substitutes for an evaluation. In any case, one can write that the evidence is 'not consistent', 'not convincing' or claims are 'unsubstantiated' and therefore 'unproven'. The same is true in experimental studies. Funds are generally not available for an independent study to track down the causes of the differences in results, so the contradictory results are juxtaposed and a draw is implied. This is a relatively cheap but effective way to neutralize or negate a positive study.

VII. Replication and failures to replicate experimental results

Independent replication of experiments is an essential criterion for acceptance of a result and one of the pillars of scientific proof. However, as we shall see below, it is very difficult to actually replicate a biological experiment. We need only remember the experience with the 'Henhouse' project run by the Office of Naval Research many years ago, when chicken eggs from different suppliers led to different effects of EMFs on chick embryo development.

While scientists generally shun replications, some failures to replicate have been analyzed and explained. The two discussed below had the earmarks of replications, but neither was. In one case, it was clearly shown by Jin et al (2000) that the investigators failed to use the precise cell type population of the original experiment. Jin et al obtained HL60 cells from the two different sources used in the papers with the contradictory results, and showed that the cells had very different growth characteristics, significantly different reactivities and reactions to EMFs. It appears that even different samples of the same cell line in the same laboratory can have different responses to EMFs. The changes that occur in tissue culture over time can result in very different responses to EMFs.

In another example, Utteridge et al (2002) published a paper in *Radiation Research* meant to test the positive results of an earlier study (Repacholi et al, 1997) that had shown a twofold increase in lymphoma in mice exposed to cell phones. They failed to replicate the findings, but even a cursory reading of the paper showed that the study was

poorly designed and executed, and was definitely not a replication. They had used a different exposure regimen and had manually handled the animals, an added stress on the mice. The cancer rate in the control group was three times the rate of the earlier study, possibly due to the handling, making it almost impossible to find any effect of cell phone exposure. There were also unusual inconsistencies in the published data, such as listing the weights of animals that had died months earlier. It is hard to see how the paper passed peer review. The Utteridge study self-destructed, and the results of the Repacholi study are still looked upon as showing a relation between RF and cancer in an animal model. However, there were scientific casualties, the peer review process of the journal and the credibility of its editors.

It may be appropriate to mention that *Radiation Research*, a journal devoted to research with ionizing radiation frequencies, has published studies that almost exclusively show no EMF effects. A quick glance at Table 1 will show that many of the 'NO effect' listings are published in that journal. It has even gone beyond the frequency range defined in its title and published 'negative' studies in the non-ionizing frequency range. The internet edition of *Microwave News* has an explanation for why this journal repeatedly publishes negative research and appears to have become so politicized on the EMF issue.

It is not unusual for scientists to deviate from an original experimental protocol when repeating an experiment. They generally view the deviations as improvements in technique. Readers who have not worked on that particular system are unlikely to focus on a small difference that does not appear to be significant. Yet, even a small difference may lead to a failed replication. Blank and Soo (2003) showed that EMF accelerated the Belousov-Zhabotinsky (BZ) reaction, which is the catalyzed oxidation of malonic acid. A subsequent study reported no effect of EMF on the BZ reaction (Sontag, 2006), in essence a failed replication. In the second study, the authors did not apply the field at the time the reactants were mixed, as in the original, but only after the reaction was well under way for about seven minutes. This time difference was critical for a reaction that responds to EMF. Other reactions had responded to EMF (Blank and Soo, 2001b; Blank, 2005) only when the field was applied at time zero, when the intrinsic chemical forces were relatively weak. The effect of EMF was even shown to vary inversely with the opposing chemical forces of an enzyme (Blank, 2005). After seven minutes, the BZ reaction was running at full speed and the applied ELF fields were not strong enough to overcome the built up chemical forces.

The above paragraph points up a critical factor often overlooked in EMF experiments. EMF is only one of the factors that can affect the rate of a biochemical reaction, and a relatively weak one in the ELF range. It appears that when an EMF accelerates charge movements associated with a reaction, the applied field competes with intrinsic forces, and the ability to see an effect of the applied EMF depends on minimizing the other forces in the system. It is obvious that an important strategy to minimize unwanted biological effects due to EMF is to maintain intrinsic forces at optimal (healthy) levels.

In the above mentioned experiments with the Na,K-ATPase (Blank, 2005), it was found

that the effect of an applied electric or magnetic field varied inversely with the activity of the enzyme, which could be changed by changing ion concentrations, temperature, inhibitors, or by the normal aging of the preparation. The effect of intrinsic activity was also observed in other systems, electron transfer from cytochrome C to cytochrome oxidase (Blank and Soo, 1998), and in the effect of temperature on the oxidation of malonic acid (Blank and Soo, 2003). Since the effect of EMF in an experiment can vary depending on the other forces acting in the system, it is important to make sure that all relevant parameters are identified and controlled. Replication of biological experiments must ensure a comparable level of intrinsic biological activity before a perturbing EMF is applied. This is especially difficult with enzyme preparations as they age.

In studies of stress protein synthesis, many factors must be considered, but the choice of cells is particularly important. Not all cells respond to EMF, and the results of many experiments have suggested ideas about critical properties that are apt to determine the response and also affect the ability to replicate an experimental result.

A quick look at Table 1 shows that tissue culture cells are more likely to show ‘**NO** effect’. That is not really surprising. Cells in tissue culture have changed significantly to enable them to live indefinitely in the unnatural conditions of a flask in a laboratory, and the changes could have made them unresponsive to EMF. The same is true of the changes in cancer cells, although some (e.g., MCF7) have responded to EMF (e.g., Liburdy et al, 1993), and in one cell line, HL60, some samples respond to EMF and others do not (Jin et al, 2000). On the other hand, the study by Czyz et al (2004) found that p53-deficient embryonic stem cells showed an increased EMF response, but the wild type did not. It is obviously difficult to make generalizations about the necessary conditions for a response to EMF when there are so many variations, and cells can undergo changes in tissue culture.

Some insight into differences between cells has been obtained from a broad study of genotoxic effects in different kinds of cells (Ivancsits et al, 2005). They found no effects with lymphocytes, monocytes and skeletal muscle cells, but did find effects with fibroblasts, melanocytes and rat granulosa cells. Other studies (e.g., Lantow et al, 2006b; Simko et al, 2006) have also found that the blood elements, such as lymphocytes and monocytes are natural cells that have not responded. From an evolutionary point of view, it may be that mobile cells can easily move away from a stress and there is little selective advantage to develop the stress response. The lack of response by skeletal muscle cells is easier to explain (Blank, 1995). It is known that cells containing fast muscle fibers do not synthesize hsp70, while those with slow fibers do. This evolutionary development protects cells from over-reacting to the high temperatures reached in fast muscles during activity.

Other natural cells listed in Table 1, such as epithelial, endothelial and epidermal cells, fibroblasts, yeast, E coli, developing chick eggs, the cells of *Drosophila*, *Sciara* and *C elegans*, have all been shown to respond. While experiments with non-responding cells have provided little information, studies of the differences between responding and non-

responding cells may be the best experimental strategy for studying the stress response mechanism. Proteomics appears to be an excellent tool for answering many of the questions about the molecular mechanisms that are activated (Leszczynski et al, 2004).

In studies of stress protein synthesis, the time course of a response must be determined. There is generally a rapid induction and a slower falloff of response, but the kinetics can be affected by many other conditions of the experiment. It is, therefore, important to look for stress proteins when they are apt to be present, and not before they have been synthesized or after the response has decayed. This may be the explanation for the inability of Cleary et al, (1997) to observe stress proteins twenty-four hours after exposure. Some additional cautions to be aware of in contemplating or evaluating a study. For example, different stresses elicit different responses, so it is important to determine which of the ~20 different stress proteins are synthesized. The most frequently studied stress proteins are hsp70 and hsp27, but others may be involved and undetected. The exposure history of a cell population must be known, since there are differences in the responses to an initial stimulus and subsequent ones. The need to provide shielding for cells becomes far more complicated when they respond to RF as well as ELF fields and one must insure no pre-exposure.

Obviously, many experiments must be done to determine the optimal conditions for the study of a particular system. This does not shift the burden of proof to those unable to find an effect, but it adds weight to the cautions generally voiced in papers that state their failure to observe stress proteins ‘under our experimental conditions’. Those words mean just that, and not that stress proteins were absent.

An experiment on EMF stimulation of cell growth that has almost disappeared from the EMF literature is the work of Robert Liburdy (Liburdy et al, 1993). He reported that weak 60Hz fields can interfere with the ability to inhibit growth in MCF7 breast cancer cells. This finding has been replicated six times, but the original experiment and its replications have been ignored by many health oriented scientists (Liburdy, 2003), including the recent WHO review (BEMS Supplement 7, 2005). Even breast cancer researchers (e.g., Loberg et al, 1999), who have not been directly involved in the EMF debate, appear to be totally unaware of results showing the ability of weak 60Hz fields to affect cancer cell growth. It is shocking when an EMF research review by a presumably scientifically neutral WHO fails to even mention any of the papers that offers insight into the mechanism of a devastating disease that is so prevalent in the population (Blank and Goodman, 2006). Let us not forget the asymmetry in scientific proof (Popper, 1959), where a single reproducible harmful effect would outweigh all the negative results. The many replications of the Liburdy experiment have given us a crucial finding regarding the question of EMF risk, and they cannot be ignored.

VIII. A critical look at a recent review of the stress response

The earlier discussion of non-scientific influences in the design and presentation of the results of EMF research serves as an introduction to a critical look at the recent review on

RF and the stress response by Cotgreave (2005) 'with contributions of the Forschungsgemeinschaft Funk'. I agree with the major conclusion of the review, the need for more research on the stress response with better controls. However, Cotgreave was highly selective in his omission of papers on ELF and stress proteins. Given that there are many relevant ELF papers reporting effects on stress proteins at non-thermal levels, this omission results in significant under-reporting of what is scientifically established. These obvious and scientifically questionable omissions were used to cast doubt on the ability of RF to have a significant biological effect, at a time when much evidence pointed in the opposite direction.

Cotgreave stated correctly that RF is pleiotropic (produces more than one gene effect) for many regulatory events, in addition to the stress response. That observation comes as no surprise to biologists who know that cellular systems are interconnected and that the complexity of the signaling pathways resembles that of the old interlinked intermediary metabolism charts. It is also no surprise to those familiar with early papers on EMFs, which showed activation of genes such as *c-myc* (Goodman and Shirley-Henderson, 1991; Lin et al, 1994;1996) and *c-fos* (Rao and Henderson, 1996) at about the same time the EMF stress response was first described (Blank et al, 1994; Goodman et al, 1994). The EMF stimulated synthesis of many proteins (Goodman and Henderson, 1988) and the binding of specific transcription factors AP-1, AP-2 and SP-1 were also previously described (Lin et al, 1998).

By highlighting the previously known pleiotropic nature of the EMF response, Cotgreave played down the role of the stress response as a protective mechanism. Had he analyzed the biological implications of the many genes activated, he could have pointed to evidence from proteomics and gene analysis that there is a relevant pattern to the pleiotropism. Kültz (2005) recently summarized the evidence that specific groups of genes are activated along with stress genes across the biological spectrum. It is of particular interest to the EMF discussion that this 'universally conserved proteome' consists largely of genes involved in sensing and repairing damage to DNA and proteins, evidence that the stress response is a reaction to molecular damage across the biological spectrum. The stress response is one of many stimulated by RF, but other parts of the response also show evidence of damage control in reaction to an EMF.

By limiting the scope of his review to effects of RF, Cotgreave overlooked much that is relevant to understanding the effects of EMFs. That was a bit like writing a review on the physiological effects of alcohol and limiting the discussion to scotch whiskey. The EM spectrum is continuous and its divisions arbitrary, so there is no good reason to limit the discussion to RF when living cells are activated and synthesize stress proteins in both RF and ELF ranges (Blank and Goodman, 2004a). Furthermore, emissions from cell phones include both RF and ELF frequencies (Linde and Mild, 1997; Jokela, 2004; Sage et al, 2007). The bulk of the original research on EMFs and the stress response was done using ELF (see review by Goodman and Blank, 1998). ELF studies also led to information about the DNA consensus sequence sensitive to EMFs that differs from the 'heat shock' consensus sequence (Lin et al, 1999). This is a critical piece of molecular evidence showing the difference between thermal and non-thermal responses. Cotgreave described

the heat shock consensus sequence, but not the EMF consensus sequence or the experiments in which such sequences were transferred and retained sensitivity to an EMF (Lin et al, 2001). For any insight into EMF-DNA interaction, it was absolutely essential to describe the molecularly based biological sensitivity to EMFs, inherent in DNA structure, that differs from thermal sensitivity and that can be manipulated.

More importantly, by considering both ELF and RF responses, it becomes obvious that the practice of describing EMF 'dose' in terms of SAR is meaningless for the stress response (Blank and Goodman, 2004a). The research on ELF stimulated stress response has shown unequivocally that SAR at the threshold is many orders of magnitude lower than in the RF range. The separation of thermal and non-thermal mechanisms had already been shown by Mashevich et al (2002), where chromosomal damage observed under RF in lymphocytes was not seen when the cells were exposed to elevated temperatures. The importance of non-thermal mechanisms was also made clear in the experiments of Bohr and Bohr (2000) in a much simpler biochemical system, showing that both denaturation and renaturation of β -lactoglobulin are accelerated by microwave EMF, and by de Pomerai et al (2003), who showed that microwave radiation causes protein aggregation without bulk heating. These as well as the ELF enzyme kinetics studies listed in Table 2 should have indicated that EMFs can cause changes in molecular structure without requiring heating.

Cotgreave overlooked a similarity between electric and magnetic ELF stimulation of DNA and endogenous electric stimulation of protein synthesis. Blank (1995) had reviewed this effect in striated muscle, and recently Laubitz et al (2006) showed that myoelectrical activity in the gut can trigger heat shock response in *E coli* and Caco-2 cells. The mechanism in striated muscle is well known. Body builders stimulate muscle activity to increase muscle mass, and biologists have known that the electric fields associated with muscle action potentials stimulate the synthesis of muscle proteins. The particular proteins synthesized appear to be related to the frequency of the action potentials, and one can even change the protein composition of a muscle by changing the frequency of the action potentials (Pette and Vrbova, 1992). Under normal physiological conditions, the action potentials along the muscle membrane drive currents across the DNA in nuclei adjacent to the membrane. The estimated magnitude of electric field, $\sim 10\text{V/m}$, provides a large safety margin in muscle, since fields as low as 3mV/m stimulate biosynthesis in HL60 cells (Blank et al, 1992). The fact that a physiological mechanism links electric stimulation to protein synthesis suggests that EMF can cause stress protein synthesis by a similar mechanism.

As a matter of proper scholarly attribution "heat shock" was first described in *Drosophila* by Ritossa (1962), and the first description of stress response due to EMF was in back-to-back papers showing similar protein distributions stimulated by temperature and ELF (Blank et al, 1994), and that both stimuli resulted in proteins that reacted with the same specific antibody for the stress protein hsp70 (Goodman et al, 1994). The ability of power frequency fields to alter RNA transcription patterns had been reported even earlier by Goodman et al (1983).

The above discussion acknowledges that Cotgreave's review was a positive contribution that summarized much useful information, but one that failed to properly assess the state of knowledge in EMF stress protein research. He gave the impression that much of the information was tenuous and that the thermal mechanism was the only one to consider. This may be his point of view and that of co-contributor, Forschungsgemeinschaft Funk. However, at the very least, he should have incorporated relevant research on stimulation of the stress response by non-thermal EMFs. The ELF data have convinced many to reject the paradigm of thermal effects only. A reader would have learned more about the stress response had the author devoted more space to the ELF papers than to papers on something called 'athermal heating'.

IX. Rethinking EMF safety in a biology context

Studies of the stress response in different cells under various conditions have enabled us to characterize the molecular mechanisms by which cells respond to EMF and their effects on health risk. That information can now correct assumptions about biological effects of EMF, and establish a scientific basis for new safety standards.

In setting standards, it is essential that basic findings in all relevant research areas are taken into account. Relevance is not subjective. It is determined by whether a study adds to our knowledge of how cells react to EMF, and this criterion determined inclusion of the references in Table 1. The criteria for the references in the IEEE list were not focused on the molecular biology of cellular responses that illuminate disease mechanisms, but were based on such assumptions as arbitrarily defined divisions of the spectrum, on thermal responses only, etc. It is therefore not surprising that many relevant studies were omitted in the IEEE literature review. Fewer than one quarter of the references listed in Table 1 appear in the IEEE list. The result of having omitted many EMF studies, including those on the stress response, is that many research results have not been utilized in setting EMF safety standards. A careful examination of basic assumptions will show that the omissions are crucial and that they indicate an urgent need to reconsider the entire basis for EMF safety standards. Here in bold are the assumptions, followed by the re-evaluations:

- **Safety standards are set by division of the EM spectrum.** It may come as a surprise to the engineers and physicists who set up the divisions of the EM spectrum, but biology does not recognize EM spectrum divisions. The same biological reaction can be stimulated in more than one subdivision of the EM spectrum. The arbitrarily defined divisions of the spectrum do not in any way confine the reactions of cells to EMF, and ELF studies do indeed contribute to an understanding of how cells respond to RF. This was discussed in the critique of Cotgreave's (2005) review. This area clearly demands immediate attention. People are getting ELF and RF simultaneously from the same device, and they are being protected from thermal effects only. This ignores the potentially harmful

effects from non-thermal ELF and RF discussed next.

- **EMF standards are based on the assumption that only ionizing radiation causes chemical change.** The stress response in both ELF and RF ranges has shown that non-ionizing radiation also causes chemical change. Several additional examples of EMF stimulated chemical change in the ELF range are listed in Table 2.

- **EMF standards are based on the assumption that non-ionizing EMF only causes damage by heating (i.e., damage by thermal effects only).** Research on the stress response in the ELF range has shown that a thermal response to a rise in temperature and the non-thermal response to EMF are associated with different DNA segments of the same gene. Both the thermal and the non-thermal mechanisms are natural responses to potential damage. Furthermore, the non-thermal stress response can occur in both the ELF and RF ranges. Other non-thermal effects of EMF have been demonstrated, e.g., acceleration of electron transfer reactions and DNA strand breaks.

- **Safety limits in the non-ionizing range are in terms of rate of heating (SAR).** The above described effects occur below the thermal safety limits in the non-ionizing range, so the safety limits provide no protection against non-thermal damage. Safety limits must include non-thermal effects.

X. Summary

It is generally agreed that EMF safety standards should be based on science, yet recent EMF research has shown that a basic assumption used to determine EMF safety is not valid. The safety standard assumes that EMF causes biological damage only by heating, but cell damage occurs in the absence of heating and well below the safety limits. This has been shown in the many studies, including the cellular stress response where cells synthesize stress proteins in reaction to potentially harmful stimuli in the environment, including EMF. The stress response to both the power (ELF) and radio (RF) frequency ranges shows the inadequacy of the thermal (SAR) standard.

The same mechanism is stimulated in both ranges, but in the ELF range, where no heating occurs, the energy input rate is over a billion times lower than in the RF range.

The stress response is a natural defense mechanism activated by molecular damage caused by environmental forces. The response involves activation of DNA, i.e., stimulating stress genes as well as genes that sense and repair damage to DNA and proteins. Scientific research has identified specific segments of DNA that respond to EMF and it has been possible to move these specific segments of DNA and transfer the sensitivity to EMF. At high EMF intensities, the interaction with DNA can lead to DNA strand breaks that could result in mutation, an initiating step in the development of cancer.

Scientific research has shown that ELF/RF interact with DNA to stimulate protein synthesis, and at higher intensities to cause DNA damage. The biological thresholds (field strength, duration) are well below current safety limits. To be in line with EMF research, a biological standard must replace the thermal (SAR) standard, which is fundamentally flawed. EMF research also indicates a need for protection against the cumulative biological effects stimulated by EMF across the EM spectrum.